PATENT APPLICATION

# RECOMBINANT CHALCOMYCIN POLYKETIDE SYNTHASE AND MODIFYING GENES

## STATEMENT OF GOVERNMENT INTEREST

**[0001]** Subject matter disclosed in this application was made, in part, with government support under NIH Grant No. R43 CA AI50305. As such, the United States government may have certain rights in this invention.

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0002]** The present application claims benefit of U.S. provisional patent application nos. 60/405,194 (filed 21 August 2002); 60/420,994 (filed 24 October 2002); and 60/493,966 (filed 8 August 2003) each of which is incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

**[0003]** The invention relates to recombinant polynucleotides that encode polypeptides or domains of the chalcomycin polyketide synthase gene cluster. Accordingly, the present invention is directed to the production of chalcomycin PKS enzymes, to polynucleotides that encode such enzymes, and to host cells that contain such polynucleotides. Further enhancements in the biological activities of chalcomycin and other polyketides, through production of derivatives thereof, is also made possible according to the practice of the invention by providing P450 hydroxylases that provide attachment points on the polyketide molecule for further modifications. Thus the present invention relates to the fields of molecular biology, chemistry, recombinant DNA technology, medicine, animal health, and agriculture.

## BACKGROUND OF THE INVENTION

**[0004]** Polyketides represent a large family of diverse compounds synthesized from 2 carbon units through a series of condensations and subsequent modifications. Polyketides occur in many types of organisms including fungi and mycelial bacteria, in particular the actinomycetes. An appreciation for the wide variety of polyketide structures and for their biological activities,

may be gained upon review of the extensive art, for example, published PCT Patent Publication WO 95/08548 and United States Patent Nos. 5,672,491 and 6,303,342

**[0005]** Polyketides are synthesized in nature by polyketide synthases ("PKS"). The Type I or modular PKS comprise a set of separate catalytic active sites; each active site is termed a "domain", and a set thereof is termed a "module". One module exists for each cycle of carbon chain elongation and modification. Figure 9 of aforementioned WO95/08548 depicts a typical Type I PKS, in this case 6-deoxyerythronolide B synthase ("DEBS"), which is involved in the production of erythromycin. Six separate modules, each catalyzing a round of condensation and modification of a 2-carbon unit, are present in DEBS. The number and type of catalytic domains that are present in each module varies based on the needed chemistry, and the total of 6 modules is provided on 3 separate polypeptides (designated DEBS-1, DEBS-2, and DEBS-3, with 2 modules per each polypeptide). Each of the DEBS polypeptides is encoded by a separate open reading frame (gene), see Caffrey *et al.*, *FEBS Letters*, 304, pp. 205, 1992. DEBS provides a representative example of a Type I PKS. In DEBS, modules 1 and 2 reside on DEBS-1, modules 3 and 4 on DEBS-2, and modules 5 and 6 on DEBS-3, wherein module 1 is defined as the first module to act on the growing polyketide backbone, and module 6 the last.

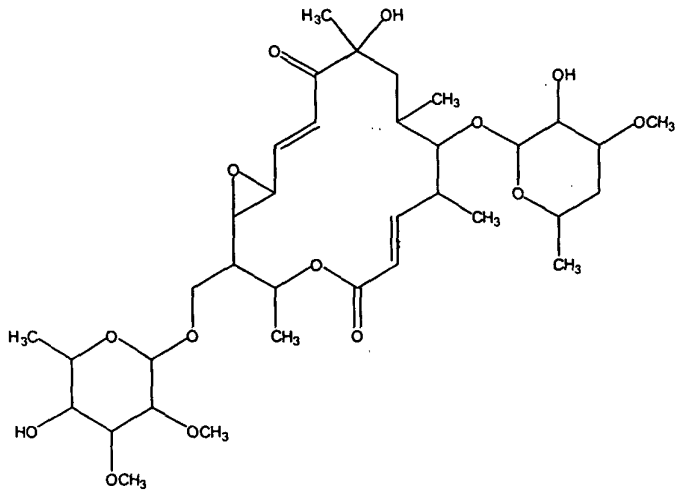
**[0006]** The minimal PKS module is typified by module 3 of DEBS which contains a ketosynthase ("KS") domain, an acyltransferase ("AT") domain, and an acyl carrier protein ("ACP") domain. These three enzyme activities are sufficient to activate a 2-carbon extender unit and attach it to the growing polyketide molecule. Additional domains that may be included in a module relate to reactions other than the actual condensation, and include domains for a ketoreductase activity ("KR"), a dehydratase activity ("DH"), and an enoylreductase activity ("ER"). With respect to DEBS-1, the first module thereof also contains an additional set of the AT and ACP activities because that module catalyzes initial condensation, and so begins with a "loading domain" (sometimes referred to as a loading module) that contains an AT and ACP, that bind the starter unit. The "finishing" of the 6-deoxyerythronolide molecule is regulated by a thioesterase activity ("TE") in module 6 that catalyzes cyclization of the macrolide ring during release of the product of the PKS.

**[0007]** PKS genes can be engineered in a variety of ways to achieve biosynthesis of polyketides. For instance, PKS genes can be inserted into a heterologous host to make a polyketide in a host that does not make it naturally. Polyketides can also be made by hybrid or

otherwise altered PKSs or polyketide biosynthetic gene clusters. Also, polyketides can be overproduced by increasing the pools of available starting polyketide biosynthetic precursors and by other means. See U.S. Pat. Nos. 5,672,491; 5,962,290; 6,080,555; 6,391,594; and 6,221,641 and PCT Patent Publications 00/47724, 01/27306, and 01/31035.

**[0008]** Chalcomycin is a 16-membered macrolide antibiotic produced by some strains of *Streptomyces bikiniensis* and possesses a broad spectrum of antimicrobial activity. Certain naturally occurring derivatives of chalcomycin produced by other *Streptomyces* organisms also have antimicrobial activity. For instance, the 8-deoxy chalcomycin derivative produced by *Streptomycin hirsutus* has antimicrobial activity against gram-positive bacteria. Chalcomycin has two modifying sugar molecules, D-mycinose and D-chalcose, the former being subject to post-glycosylation modification by O-methylation at two positions. For additional information regarding chalcomycin, see Woo, P.W.K. *et al.*, *J.A.C.S.*, 1962, 84, 1512; 1964, 86, 2724; 2726; Celmer, W.D., *J.A.C.S.*, 1965, 87, 1801; Omura, S. *et al.*, *J.A.C.S.*, 1975, 97, 4001; Neszmelyi, A. *et al.*, *Chem. Comm.*, 1976, 97; Jardim, M.E. *et al.*, *Int. J. Mass Spectrom. Ion Phys.*, 1983, 48, 189; Hauske, J.R. *et al.*, *J.O.C.*, 1986, 51, 2808; Kim, S.D. *et al.*, *J. Antibiot.*, 1996, 49, 955; Woo, P.W.K. *et al.*, *Tetrahedron*, 1996, 52, 3857 and Goo, Y.M. *et al.*, *J. Antibiot.*, 1997, 50, 85.

**[0009]** The chemical structure of chalcomycin, shown without stereochemistry, is provided by formula I below.




Formula I

**[0010]** Chalcomycin is synthesized by a Type I or modular PKS and modification enzymes. Post-PKS modification reactions include P450 oxidation at three sites to add hydroxyl groups

and glycosylation at the C5 hydroxyl to add D-chalcose, and at the C20 hydroxyl to add allose, which is then methylated at two positions to yield D-mycinose.

**[0011]** There is a need for recombinant nucleic acids, host cells, and methods of using those host cells to produce polyketides including but not limited to chalcomycin and chalcomycin analogs. These and other needs are met by the materials and methods provided by the present invention.

## SUMMARY OF THE INVENTION

**[0012]** The present invention provides recombinant nucleic acids encoding polyketide synthases and polyketide modification enzymes. The recombinant nucleic acids of the invention are useful in the production of polyketides, including but not limited to chalcomycin and chalcomycin analogs and derivatives in recombinant host cells. The biosynthesis of chalcomycin is performed by a modular PKS and polyketide modification enzymes. The chalcomycin synthase is made up of several proteins, each having one or more modules. The modules have domains with specific synthetic functions.

**[0013]** The present invention also provides domains and modules of the chalcomycin PKS and corresponding nucleic acid sequences encoding them and/or parts thereof. Such compounds are useful in the production of hybrid PKS enzymes and the recombinant genes that encode them.

**[0014]** The present invention also provides modifying genes of chalcomycin biosynthetic gene cluster in recombinant form, including but not limited to isolated form and incorporated into a vector or the chromosomal DNA of a host cell. The present invention also provides recombinant P450 hydroxylases that provide hydroxyl attachment points useful for further chemical modification. The P450 hydroxylases of the present invention include ChmHI, ChmPI and ChmPII hydroxylases.

**[0015]** The present invention also provides recombinant host cells that contain the nucleic acids of the invention. In one embodiment, the host cell provided by the invention is a *Streptomyces* host cell that produces a chalcomycin modification enzyme and/or a domain, module, or protein of the chalcomycin PKS. Methods for the genetic manipulation of *Streptomyces* are described in Kieser *et al*, "Practical Streptomyces Genetics," The John Innes Foundation, Norwich (2000), which is incorporated herein by reference in its entirety.

[0016] Accordingly, there is provided a recombinant PKS wherein at least 10, 15, 20, or more consecutive amino acids in one or more domains of one or more modules thereof are derived from one or more domains of one or more modules of chalcomycin polyketide synthase. Preferably at least an entire domain of a module of chalcomycin synthase is included. Representative chalcomycin PKS domains useful in this aspect of the invention include, for example, KR, DH, ER, AT, ACP and KS domains. In one embodiment of the invention, the PKS is assembled from polypeptides encoded by DNA molecules that comprise coding sequences for PKS domains, wherein at least one encoded domain corresponds to a domain of chalcomycin PKS. In such DNA molecules, the coding sequences are operably linked to control sequences so that expression therefrom in host cells is effective. In this manner, chalcomycin PKS coding sequences or modules and/or domains can be made to encode PKS to biosynthesize compounds having antibiotic or other useful bioactivity other than chalcomycin.

[0017] These and other aspects of the present invention are described in more detail in the Detailed Description of the Invention, below.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 illustrates the structure of the chalcomycin PKS biosynthetic gene cluster, and cosmids pKOS146.185.1 and pKOS146.185.10, which contain insert DNA encompassing the chalcomycin PKS gene cluster and associated modification enzyme genes. Abbreviations: ACP, acyl carrier protein; *chm*, chalcomycin gene; Orf, open reading frame.

[0019] Figure 2 shows proposed pathways for post-PKS modification of the chalcomycin-spiramycin hybrid PKS macrolide product.

## DETAILED DESCRIPTION OF THE INVENTION

[0020] The invention provides recombinant materials for the production of polyketides. In an aspect, the present invention provides recombinant nucleic acids encoding polyketide synthases that contain all or a portion of the chalcomycin PKS. The biosynthesis of chalcomycin is performed by a modular PKS and modification enzymes. The chalcomycin synthase is made up of five proteins, each having one or more modules, each module comprising domains with specific synthetic functions. Thus, the present invention also provides the domains and modules

of the chalcomycin PKS and corresponding nucleic acid sequences encoding them in recombinant form.

**[0021]** Modifying genes of the chalcomycin biosynthetic gene cluster are also provided, including but not limited to the genes for the ChmHI, ChmPI and ChmPII P450 hydroxylases that provide hydroxyl attachment points useful for further chemical modification.

**[0022]** Methods and host cells for using these genes to produce or modify a polyketide in recombinant host cells are also provided.

**[0023]** The nucleotide sequences encoding chalcomycin PKS and modifying proteins of the present invention were isolated from *Streptomyces bikiniensis* NRRL 2737 (obtained from the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois USA). The chalcomycin PKS gene cluster and modifying genes are contained in cosmids pKOS 146.185.1 and pKOS146.185.10. The cloning and characterization of the chalcomycin PKS gene cluster is described in Example 1, *infra*. pKOS146-185.1 was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 19 February 2003, with accession number PTA-4961. pKOS146-185.10 was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 19 February 2003, with accession number PTA-4962.

**[0024]** Given the valuable properties of chalcomycin and its modifying enzymes, means to produce useful quantities thereof and derivatives or analogs of chalcomycin are valuable. Further, the chalcomycin modifying enzymes can also be used to modify other polyketides and produce derivatives thereof with enhanced solubility and/or bioactivity, for instance as antibiotics, and/or sites for further enzymatic or chemical modification. The nucleotide sequences of the chalcomycin biosynthetic gene cluster encoding chalcomycin PKS and modifying enzymes, and domains and/or modules of the PKS can be used, for example, to make antibiotics or other useful compounds in addition to chalcomycin, and in host cells in addition to *Streptomyces bikiniensis*.

**[0025]** There is a need for recombinant nucleic acids, host cells, and methods of expressing those nucleic acids in host cells resulting in production of chalcomycin and or its analogs or derivatives, and modifying enzymes, such as the cytochrome P450 hydroxylases that specifically attach hydroxyl groups on the resulting aglycone (which can then be used as attachment points

for further modifications). The modifying P450's from the chalcomycin PKS cluster of the present invention can be used to make compounds in a host that does not naturally produce such compounds. These and other needs are met by the materials and methods of the present invention

**[0026]** In one aspect of the invention, purified and isolated DNA molecules are provided that comprise one or more coding sequences for one or more domains or modules of chalcomycin synthase. Examples of such encoded domains include chalcomycin synthase KR, DH, ER, AT, ACP, and KS domains. In one aspect, the invention provides DNA molecules in which the complete set of chalcomycin PKS-encoding sequences are operably linked to expression control sequences that are effective in suitable host cells to produce chalcomycin and/or its analogs or derivatives. In one aspect, the invention provides polypeptides comprising a portion of the coding sequences for the proteins of the chalcomycin synthase.

**[0027]** Table 2 in Example 1 provides a description of genes in the chalcomycin PKS gene (i.e., SEQ ID NO:1 and subsequences encoding modules, domains and ORFs, e.g., as indicated), as well as encoded proteins (including SEQ ID. NOS: 2-43) or domains. It will be apparent from Table 2, and Figures 1 and 2, which DNA strand comprises the coding sequence for a protein (i.e., the strand having the sequence of SEQ ID NO:1, or its complement.

**[0028]** In one aspect, the invention provides an isolated or recombinant DNA molecule comprising a nucleotide sequence that encodes at least one polypeptide, alternatively at least one module, alternatively at least one domain, involved in the biosynthesis of a chalcomycin. In one aspect, the invention provides the present invention provides an isolated or recombinant DNA molecule comprising a nucleotide sequence that encodes at least one polypeptide, alternatively at least one module, alternatively at least one domain, involved in the biosynthesis of a chalcomycin. The invention also provides polypeptides comprising PKS interpolypeptide linker sequences, and polynucleotides encoding such linker sequences. Also provided by the invention are polypeptides comprising intrapolypeptide linker sequences, and polynucleotides encoding such linkers.

**[0029]** In one aspect, the invention provides an isolated or recombinant DNA molecule comprising a sequence identical or substantially similar to at least one subsequence of SEQ ID NO:1 or its complement. In an embodiment the subsequence comprises a sequence encoding a chalcomycin PKS domain or module. In an aspect, the invention provides a recombinant DNA molecule that encodes a polypeptide, module or domain derived from a chalcomycin polyketide

synthase (PKS) gene cluster. In this context, a polypeptide, module or domain is derived from a chalcomycin polyketide synthase (PKS) gene cluster when it is encoded by a DNA with substantial sequence identity to the corresponding coding region of the *S. bikiniensis* chalcomycin gene cluster. For example, in an embodiment, the DNA encoding sequence of the polypeptide, module or domain hybridizes under stringent conditions to a nucleic acid having the sequence of SEQ ID NO:1 (or its complement). Generally, such a polypeptide, module or domain is biologically active, i.e., has at least one enzymatic activity chracteristic of the polypeptide, module or domain encoded exactly by corresponding sequence of SEQ ID NO:1 or its complement. The biological activity of a polypeptide of the invention can be measured by methods well known to the art.

**[0030]** In one aspect, the invention provides the present invention provides an isolated or recombinant DNA molecule comprising a nucleotide sequence that encodes an open reading frame, module or domain having an amino acid sequence identical or substantially similar to an ORF, module or domain encoded by SEQ ID NO:1 or its complement. Generally, a polypeptide, module or domain having a sequence substantially similar to a reference sequence has substantially the same activity as the reference protein, module or domain (e.g., when integrated into an appropriate PKS framework using methods known in the art). In certain embodiments, one or more activities of a substantially similar polypeptide, module or domain are modified or inactivated as described below.

**[0031]** In one aspect, the invention provides an isolated or recombinant DNA molecule comprising a nucleotide sequence that encodes at least one polypeptide, module or domain encoded by SEQ ID NO:1, e.g., a polypeptide, module or domain involved in the biosynthesis of a chalcomycin, wherein said nucleotide sequence comprises at least 20, 25, 30, 35, 40, 45, or 50 contiguous base pairs identical to a sequence of SEQ ID NO:1 or its complement. In one aspect, the invention provides an isolated or recombinant DNA molecule comprising a nucleotide sequence that encodes at least one polypeptide, module or domain encoded by SEQ ID NO:1, e.g., a polypeptide, module or domain involved in the biosynthesis of a chalcomycin, wherein said polypeptide, module or domain comprises at least 10, 15, 20, 30, or 40 contiguous residues of a corresponding polypeptide, module or domain encoded by SEQ ID NO:1 or its complement.

**[0032]** In a related aspect, the invention provides a recombinant DNA molecule, comprising a sequence of at least about 200, optionally at least about 500, basepairs with a sequence

identical or substantially identical to a protein encoding region of SEQ ID NO:1. In an embodiment, the DNA molecule encodes a polypeptide, module or domain derived from a chalcomycin polyketide synthase (PKS) gene cluster.

**[0033]** It will be understood that SEQ ID NO:1 was determined using the inserts of pKOS 146.185.1 and pKOS146-185.10. Accordingly, the invention provides an isolated or recombinant DNA molecule comprising a sequence identical or substantially similar to a ORF encoding sequence of the insert of pKOS 146.185.1 or pKOS146-185.10.

**[0034]** Those of skill will recognize that, due to the degeneracy of the genetic code, a large number of DNA sequences encode the amino acid sequences of the domains, modules, and proteins of the chalcomycin PKS, the enzymes involved in chalcomycin modification and other polypeptides encoded by the genes of the chalcomycin biosynthetic gene cluster. The present invention contemplates all such DNAs. For example, it may be advantageous to optimize sequence to account for the codon preference of a host organism. The invention also contemplates naturally occurring genes encoding the chalcomycin PKS and tailoring enzymes that are polymorphic or other variants. In addition, it will be appreciated that polypeptide, modules and domains of the invention may comprise one or more conservative amino acid substitutions relative to the polypeptides encoded by SEQ ID NO: 1, such as, for example, conservative substitutions include aspartic-glutamic as acidic amino acids; lysine/arginine/histidine as basic amino acids; leucine/isoleucine, methionine/valine, alanine/valine as hydrophobic amino acids; serine/glycine/alanine/threonine as hydrophilic amino acids.

**[0035]** As used herein, the terms "substantial identity," "substantial sequence identity," or "substantial similarity" in the context of nucleic acids, refers to a measure of sequence similarity between two polynucleotides. Substantial sequence identity can be determined by hybridization under stringent conditions, by direct comparison, or other means. For example, two polynucleotides can be identified as having substantial sequence identity if they are capable of specifically hybridizing to each other under stringent hybridization conditions. Other degrees of sequence identity (e.g., less than "substantial") can be characterized by hybridization under different conditions of stringency. "Stringent hybridization conditions" refers to conditions in a range from about 5°C to about 20°C or 25°C below the melting temperature (Tm) of the target sequence and a probe with exact or nearly exact complementarity to the target. As used herein,

the melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands. Methods for calculating the Tm of nucleic acids are well known in the art (see, e.g., Berger and Kimmel, 1987, Methods In Enzymology, Vol. 152: Guide To Molecular Cloning Techniques, San Diego: Academic Press, Inc. and Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd Ed., Vols. 1-3, Cold Spring Harbor Laboratory). Typically, stringent hybridization conditions for probes greater than 50 nucleotides are salt concentrations less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion at pH 7.0 to 8.3, and temperatures at least about 50°C, preferably at least about 60°C. As noted, stringent conditions may also be achieved with the addition of destabilizing agents such as formamide, in which case lower temperatures may be employed. Exemplary conditions include hybridization at 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$ pH 7.0, 1 mM EDTA at 50°C (or alternatively 65°C); wash with 2×SSC, 1% SDS, at 50°C (or alternatively 0.1 - 0.2 ×SSC, 1% SDS, at 50°C or 65°C). Other exemplary conditions for hybridization include (1) high stringency: 0.1×SSPE, 0.1% SDS, 65°C.; (2) medium stringency: 0.2×SSPE, 0.1% SDS, 50° C.; and (3) low stringency: 1.0×SSPE, 0.1% SDS, 50° C. Equivalent stringencies may be achieved using alternative buffers, salts and temperatures.

**[0036]** Alternatively, substantial sequence identity can be described as a percentage identity between two nucleotide or amino acid sequences. Two nucleic acid sequences are considered substantially identical when they are at least about 70% identical, at least about 75% identical, or at least about 80% identical, or at least about 85% identical, or at least about 90% identical, or at least about 95% or 98% identical. Two amino acid sequences are considered substantially identical when they are at least about 60%, sequence identical, more often at least about 70%, at least about 80%, or at least about 90% sequence identity to the reference sequence. Percentage sequence (nucleotide or amino acid) identity is typically calculated using art known means to determine the optimal alignment between two sequences and comparing the two sequences. Optimal alignment of sequences may be conducted using the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444, by the BLAST algorithm of Altschul (1990) *J. Mol. Biol.* 215: 403-410; and Shpaer (1996) *Genomics* 38:179-191, or by the Needleham et al. (1970) *J. Mol. Biol.* 48: 443-453; and Sankoff et al.,

1983, *Time Warps, String Edits, and Macromolecules, The Theory and Practice of Sequence Comparison*, Chapter One, Addison-Wesley, Reading, MA; generally by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI; BLAST from the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov/). In each case default parameters are used (for example the BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands).

**[0037]** As used herein the term "recombinant" has its usual meaning in the art and refers to a polynucleotide synthesized or otherwise manipulated in vitro, or to methods of using recombinant polynucleotides to produce gene products in cells or other biological systems. Thus, a "recombinant" polynucleotide is defined either by its method of production or its structure. In reference to its method of production, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, a recombinant polynucleotide can be a polynucleotide made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature. Thus, for example, products made by transforming cells with any non-naturally occurring vector is encompassed, as are polynucleotides comprising sequence derived using any synthetic oligonucleotide process, as are polynucleotides from which a region has been deleted. A recombinant polynucleotide can also be a coding sequence that has been modified *in vivo* using a recombinant oligo or polynucleotide (such as a PKS in which a domain is inactivated by homologous recombination using a recombinant polynucleotide). A "recombinant" polypeptide is one expressed from a recombinant polynucleotide.

**[0038]** It will be immediately recognized by those of skill that recombinant polypeptides of the invention have a variety of uses, some of which are described in detail below, including but not limited to use as enzymes, or componants of enzymes, useful for the synthesis or modification of polyketides. Recombinant polypeptides encoded by the chalcomycin PKS gene cluster are also useful as antigens for production of antibodies. Such antibodies find use for purification of bacterial (e.g., *Streptomyces bikiniensis*) proteins, detection and typing of

bacteria, and particularly, as tools for strain improvement (e.g., to assay PKS protein levels to identify "up-regulated" strains in which levels of polyketide producing or modifying proteins are elevated) or assessment of efficiency of expression of recombinant proteins. Polyclonal and monoclonal antibodies can be made by well known and routine methods (see, e.g., Harlow and Lane, 1988, ANTIBODIES: A LABORATORY MANUAL, COLD SPRING HARBOR LABORATORY, New York; Koehler and Milstein 1075, Nature 256:495). In selecting polypeptide sequences for antibody induction, it is not to retain biological activity; however, the protein fragment must be immunogenic, and preferably antigenic (as can be determined by routine methods). Generally the protein fragment is produced by recombinant expression of a DNA comprising at least about 60, more often at least about 200, or even at least about 500 or more base pairs of protein coding sequence, such as a polypeptide, module or domain derived from a chalcomycin polyketide synthase (PKS) gene cluster. Methods for expression of recombinant proteins are well known. (See, e.g., Ausubel et al., 2002, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York.)

**[0039]** Further aspects of the invention include chimeric PKSs comprising a portion (in one embodiment at least a domain, optionally at least a module, or alternatively at least one polypeptide) from the chalcomycin PKS, and a portion (in one embodiment at least a domain, optionally at least a module, or alternatively at least a polypeptide) from one or more non-chalcomycin PKSs. For example, the invention provides (1) encoding DNA for a chimeric PKS that is substantially patterned on a non-chalcomycin producing enzyme, but which includes one or more functional domains or modules of chalcomycin PKS; (2) encoding DNA for a chimeric PKS that is substantially patterned on the chalcomycin PKS, but which includes one or more functional domains or modules of another PKS or NRPS; and (3) methods for making chalcomycin analogs and derivatives. With respect to item (1) above, examples include chimeric PKS enzymes wherein the genes for the erythromycin PKS, rapamycin PKS, tylosin PKS, and spiramycin PKS, or another PKS function as accepting genes, and one or more of the above-identified coding sequences for chalcomycin domains or modules are inserted as replacements for domains or modules of comparable function. With respect to item (2) above, examples include chimeric PKS enzymes wherein the chalcomycin PKS serves as an accepting gene, and genes for the erythromycin PKS, rapamycin PKS, tylosin PKS, and spiramycin PKS, or another PKS function as accepting genes, and one or more of the above-identified coding sequences for

chalcomycin domains or modules are inserted as replacements for domains or modules of comparable function. A partial list of sources of PKS sequences for use in making chimeric molecules, for illustration and not limitation, includes Avermectin (U.S. Pat. No. 5,252,474; MacNeil et al., 1993, Industrial Microorganisms: Basic and Applied Molecular Genetics, Baltz, Hegeman, & Skatrud, eds. (ASM), pp. 245-256; MacNeil et al., 1992, Gene 115: 119-25); Candicidin (FRO008) (Hu et al., 1994, *Mol. Microbiol.* 14: 163-72); Epothilone (U.S. Pat. No. 6,303,342); Erythromycin (WO 93/13663; U.S. Pat. No. 5,824,513; Donadio et al., 1991, *Science* 252:675-79; Cortes et al., 1990, *Nature* 348:176-8); FK-506 (Motamedi et al., 1998, *Eur. J. Biochem.* 256:528-34; Motamedi et al., 1997, *Eur. J. Biochem.* 244:74-80); FK-520 (U.S. Pat. No. 6,503,737; see also Nielsen et al., 1991, *Biochem.* 30:5789-96); Lovastatin (U.S. Pat. No. 5,744,350); Nemadectin (MacNeil et al., 1993, *supra*); Niddamycin (Kakavas et al., 1997, *J. Bacteriol.* 179:7515-22); Oleandomycin (Swan et al., 1994, *Mol. Gen. Genet.* 242:358-62; U.S. Pat. No. 6,388,099; Olano et al., 1998, *Mol. Gen. Genet.* 259:299-308); Platenolide (EP Pat. App. 791,656 ); Rapamycin (Schwecke et al., 1995, *Proc. Natl. Acad. Sci. USA* 92:7839-43); Aparicio et al., 1996, *Gene* 169:9-16); Rifamycin (August et al., 1998, *Chemistry & Biology*, 5: 69-79); Soraphen (U.S. Pat. No. 5,716,849; Schupp et al., 1995, *J. Bacteriology* 177: 3673-79); Spiramycin (U.S. Pat. No. 5,098,837); Tylosin (EP 0 791,655; Kuhstoss et al., 1996, Gene 183:231-36; U.S. Pat. No. 5,876,991). Additional suitable PKS coding sequences remain to be discovered and characterized, but will be available to those of skill (e.g., by reference to GenBank).

**[0040]** Construction of such chimeric enzymes is most effectively achieved by construction of appropriate encoding polynucleotides. In preparing modified and chimeric proteins, it is not necessary, although it may be most efficient, to replace or substitute one or more entire domains or modules of one PKS (e.g., the chalcomycin PKS or another PKS) with an entire domain or module of a different PKS (e.g., the chalcomycin PKS or another PKS). Rather, peptide subsequences of a PKS domain or module that correspond to a peptide subsequence in an accepting domain or module, or which otherwise provide useful function, may be used as replacements. Accordingly, appropriate encoding DNAs for construction of such chimeric PKS include those that encode at least 10, 15, 20 or more amino acids of a selected chalcomycin domain or module. Recombinant methods for manipulating modular PKS genes to make chimeric PKS enzymes are described in U.S. Patent Nos. 5,672,491; 5,843,718; 5,830,750; and

5,712,146; and in PCT publication Nos. 98/49315 and 97/02358. A number of genetic engineering strategies have been used with DEBS to demonstrate that the structures of polyketides can be manipulated to produce novel natural products, primarily analogs of the erythromycins (see the patent publications referenced supra and Hutchinson, 1998, *Curr Opin Microbiol.* 1:319-329, and Baltz, 1998, *Trends Microbiol. 6*:76-83).

**[0041]** The invention methods may be directed to the preparation of an individual polyketide. The polyketide may or may not be novel, but the method of preparation permits a more convenient or alternative method of preparing it. The resulting polyketides may be further modified to convert them to other useful compounds. Examples of chemical structures of sixteen-membered macrolides that can be made using the materials and methods of the present invention are described in PCT Patent Publication WO 02/32916; U.S. Patent Application US20020128213A (app. no. 09/969,177); and copending U.S. provisional patent application no. 60/493,966.

**[0042]** The recombinant DNAs and DNA vectors of the inventions can also be used to make "libraries" of polyketides. Generally, members of these polyketide libraries may themselves be novel compounds, and the invention further includes novel polyketide members of these libraries. Regardless of the naturally occurring PKS gene used as an acceptor, the invention provides libraries of polyketides by generating modifications in, or using a portion of, the chalcomycin PKS so that the protein complexes produced have altered activities in one or more respects, and thus produce polyketides other than the natural product of the PKS. Novel polyketides may thus be prepared, or polyketides in general prepared more readily, using this method. By providing a large number of different genes or gene clusters derived from a naturally occurring PKS gene cluster, each of which has been modified in a different way from the native cluster, an effectively combinatorial library of polyketides can be produced as a result of the multiple variations in these activities.

**[0043]** As noted, in one aspect the invention provides recombinant PKS wherein at least 10, 15, 20, or more consecutive amino acids in one or more domains of one or more modules thereof are derived from one or more domains of one or more modules of chalcomycin polyketide synthase. A polyketide synthase "derived from" a naturally occurring PKS contains the scaffolding encoded by all the portion employed of the naturally occurring synthase gene, contains at least two modules that are functional, and contains mutations, deletions, or

replacements of one or more of the activities of these functional modules so that the nature of the resulting polyketide is altered. This definition applies both at the protein and genetic levels. Particular embodiments include those wherein a KS, AT, KR, DH, or ER has been deleted or replaced by a version of the activity from a different PKS or from another location within the same PKS, and derivatives where at least one noncondensation cycle enzymatic activity (KR, DH, or ER) has been deleted or wherein any of these activities has been added or mutated so as to change the ultimate polyketide synthesized.

**[0044]** There are at least five degrees of freedom for constructing a polyketide synthase in terms of the polyketide that will be produced. First, the polyketide chain length will be determined by the number of modules in the PKS. Second, the nature of the carbon skeleton of the PKS will be determined by the specificities of the acyl transferases which determine the nature of the extender units at each position -- e.g., malonyl, methyl malonyl, methoxy malonyl, or ethyl malonyl, etc. Third, the loading domain specificity will also have an effect on the resulting carbon skeleton of the polyketide. Fourth, the oxidation state at various positions of the polyketide will be determined by the dehydratase and reductase portions of the modules. This will determine the presence and location of ketone, alcohol, alkene or alkane substituents at particular locations in the polyketide. Fifth, the stereochemistry of the resulting polyketide is a function of three aspects of the synthase. The first aspect is related to the AT/KS specificity associated with substituted malonyls as extender units, which affects stereochemistry only when the reductive cycle is missing or when it contains only a ketoreductase since the dehydratase would abolish chirality. Also, the specificity of the ketoreductase will determine the chirality of the corresponding hydroxyl group. Also, the enoyl reductase specificity for substituted malonyls as extender units will influence the result when there is a complete KR/DH/ER available.

**[0045]** As can be appreciated by those skilled in the art, polyketide biosynthesis can be manipulated to make a product other than the product of a naturally occurring PKS biosynthetic cluster. For example, AT domains can be altered or replaced to change specificity. For example, and not limitation, the AT domain of chalcomycin module 0 (loading domain) can be replaced by an AT with specificity for methylmalonyl-CoA to produce chalcomycin derivatives with a C-15 ethyl group in place of the C-15 methyl group. The variable domains within a module can be deleted and or inactivated or replaced with other variable domains found in other modules of the same PKS or from another PKS. See e.g., Katz & McDaniel, *Med Res Rev* 19: 543-558 (1999)

and WO 98/49315. Similarly, entire modules can be deleted and/or replaced with other modules from the same PKS or another PKS. See e.g., Gokhale et al., *Science* 284:482 (1999) and WO 00/47724. For example, and not limitation, 3-hydroxy derivatives of chalcomycin can be produced using a modified chalcomycin PKS in which module 7 of the chalcomycin PKS is replaced by module 7 of the tylosin PKS (optionally with appropriate linker modifications). Similarly, protein subunits of different PKSs also can be mixed and matched to make compounds having the desired backbone and modifications. For example, subunits of 1 and 2 (encoding modules 1-4) of the pikromycin PKS were combined with the DEBS3 subunit to make a hybrid PKS product (see Tang et al., *Science*, 287: 640 (2001), WO 00/26349 and WO 99/6159). Also see Examples, below.

**[0046]** Mutations can be introduced into PKS genes such that polypeptides with altered activity are encoded. Polypeptides with "altered activity" include those in which one or more domains are inactivated or deleted, or in which a mutation changes the substrate specificity of a domain, as well as other alterations in activity. Mutations can be made to the native sequences using conventional techniques. The substrates for mutation can be an entire cluster of genes or only one or two of them; the substrate for mutation may also be portions of one or more of these genes. Techniques for mutation include preparing synthetic oligonucleotides including the mutations and inserting the mutated sequence into the gene encoding a PKS subunit using restriction endonuclease digestion. (See, *e.g.*, Kunkel, T.A. *Proc Natl Acad Sci USA* (1985) 82:448; Geisselsoder *et al. BioTechniques* (1987) 5:786.) Alternatively, the mutations can be effected using a mismatched primer (generally 10-20 nucleotides in length) that hybridizes to the native nucleotide sequence (generally cDNA corresponding to the RNA sequence), at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located. (See Zoller and Smith, *Methods in Enzymology* (1983) 100:468). Primer extension is effected using DNA polymerase. The product of the extension reaction is cloned, and those clones containing the mutated DNA are selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations. (See, *e.g.*, Dalbie-McFarland *et al. Proc Natl Acad Sci USA* (1982) 79:6409). PCR mutagenesis can also be used for effecting the desired mutations.

**[0047]** Random mutagenesis of selected portions of the nucleotide sequences encoding enzymatic activities can be accomplished by several different techniques known in the art, e.g., by inserting an oligonucleotide linker randomly into a plasmid, by irradiation with X-rays or ultraviolet light, by incorporating incorrect nucleotides during *in vitro* DNA synthesis, by error-prone PCR mutagenesis, and by preparing synthetic mutants or by damaging plasmid DNA *in vitro* with chemicals. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, agents which damage or remove bases thereby preventing normal base-pairing such as hydrazine or formic acid, analogues of nucleotide precursors such as nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine intercalating agents such as proflavine, acriflavine, quinacrine, and the like. Generally, plasmid DNA or DNA fragments are treated with chemicals, transformed into *E. coli* and propagated as a pool or library of mutant plasmids.

**[0048]** In addition to providing mutated forms of regions encoding enzymatic activity, regions encoding corresponding activities from different PKS synthases or from different locations in the same PKS synthase can be recovered, for example, using PCR techniques with appropriate primers. By "corresponding" activity encoding regions is meant those regions encoding the same general type of activity -- e.g., a ketoreductase activity in one location of a gene cluster would "correspond" to a ketoreductase-encoding activity in another location in the gene cluster or in a different gene cluster; similarly, a complete reductase cycle could be considered corresponding -- e.g., KR/DH/ER could correspond to KR alone.

**[0049]** If replacement of a particular target region in a host polyketide synthase is to be made, this replacement can be conducted *in vitro* using suitable restriction enzymes or can be effected *in vivo* using recombinant techniques involving homologous sequences framing the replacement gene. One such system involving plasmids of differing temperature sensitivities is described in PCT application WO 96/40968. Another useful method for modifying a PKS gene (e.g., making domain substitutions or "swaps") is a RED/ET cloning procedure developed for constructing domain swaps or modifications in an expression plasmid without first introducing restriction sites. The method is related to ET cloning methods (see, Datansko & Wanner, 2000, Proc. Natl. Acad. Sci. U.S.A. 97, 6640-45; Muyrers et al, 2000, Genetic Engineering 22:77-98). The RED/ET cloning procedure is used to introduce a unique restriction site in the recipient plasmid at the location of the targeted domain. This restriction site is used to subsequently linearize the recipient plasmid in a subsequent ET cloning step to introduce the modification.

This linearization step is necessary in the absence of a selectable marker, which cannot be used for domain substitutions. An advantage of using this method for PKS engineering is that restriction sites do not have to be introduced in the recipient plasmid in order to construct the swap, which makes it faster and more powerful because boundary junctions can be altered more easily.

**[0050]** In one embodiment, the invention provides a chimeric PKS in which one of more polypeptides are derived from a chalcomycin PKS polypeptide, and one or more peptides are derived from one or more non-chalcomycin PKS(s) that, like the chalcomysin PKS, produces a 16-membered macrolide. Examples of PKS(s) that produces a 16-membered macrolide include, for example, the tylosin PKS, the spiramycin PKS, the niddamycin PKS, and the mycinamicin PKS. All the currently known PKSs for 16-membered macrolides consists of five large polypeptides encoded by colinear genes in a single operon. The arrangement of modules on these polypeptides is conserved. Thus, for known 16-membered macrolide PKSs, the first polypeptide has a loading module and two extender modules, the second a single extender module, the third two extender modules, the fourth a single extender module, and the fifth a single extender module followed by a thioesterase domain. The different aglycone core structures produced by different 16-membered macrolide PKSs is due to differences in the catalytic domains within each of these modules.

**[0051]** As is illustrated in the examples, below, new hybrid 16-membered macrolides can be made by expressing combinations of PKS polypeptides from different sources in a suitable host. The hybrid PKS produces hybrid polyketides that, optionally can be further modified by the post-PKS tailoring enzymes present within the host. See Examples, *infra.*

**[0052]** By expressing particular combinations of these PKS polypeptides one can produce molecules with desired combinations of structural features based on, for example, the macrolactone structural features specified by each of the five polypeptides of different 16-membered macrolide PKSs as shown in Table 1, below. As noted, by expressing particular combinations of these PKS polypeptides one can produce molecules with desired combinations of structural features. Although, as described in the Examples and Table 1, selection of particular combinations of polypeptides provides a level of predictability as to the products formed by the hybrid PKS, the invention is not limited to any particular combinations or structures "predicted" by the table.

**Table 1**

| PKS | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| TylG | 15-ethyl<br>14-methyl<br>12,13-ene<br>12-methyl | 10,11-ene<br>10-H | 9-keto<br>8-methyl<br>7-methylene<br>6-ethyl | 5-hydroxy<br>4-methyl | 3-hydroxy<br>2-H |
| SrmG | 15-methyl<br>14-H<br>12,13-ene<br>12-H | 10,11-ene<br>10-H | 9-keto<br>8-methyl<br>7-methylene<br>6-ethyl | 5-hydroxy<br>4-methoxy | 3-hydroxy<br>2-H |
| ChmG | 15-methyl<br>14-methyl<br>12,13-ene<br>12-H | 10,11-ene<br>10-H | 9-keto<br>8-methyl<br>7-methylene<br>6-methyl | 5-hydroxy<br>4-methyl | 3-keto<br>2-H |

**[0053]** In one embodiment, the components of the chimeric PKS are arranged onto polypeptides having interpolypeptide linkers that direct the assembly of the polypeptides into the functional PKS protein, such that it is not required that the PKS have the same arrangement of modules in the polypeptides as observed in natural PKSs. Suitable interpolypeptide linkers to join polypeptides and intrapolypeptide linkers to join modules within a polypeptide are described in PCT publication WO 00/47724.

**[0054]** In one embodiment of the invention, the components of the PKS are arranged into five polypeptides similarly to natural PKS proteins involved in the biosynthesis of tylactone, platenolide, and the like. Thus, for example, the first polypeptide comprises the loading domain, first and second extender modules, and a C-terminal interpolypeptide linker region suitable for interaction with the second polypeptide. The second polypeptide comprises an N-terminal interpolypeptide linker region suitable for interaction with the first polypeptide, the third extender module, and a C-terminal interpolypeptide linker region suitable for interaction with the third polypeptide. The third polypeptide comprises an N-terminal interpolypeptide linker region suitable for interaction with the second polypeptide, the fourth and fifth extender modules, and a C-terminal interpolypeptide linker region suitable for interaction with the fourth polypeptide. The fourth polypeptide comprises an N-terminal interpolypeptide linker region suitable for interaction with the third polypeptide, the sixth extender module, and a C-terminal interpolypeptide linker region suitable for interaction with the fifth polypeptide. The fifth polypeptide comprises an N-terminal interpolypeptide linker region suitable for interaction with the fourth polypeptide, the seventh extender module, and the terminal thioesterase domain.

[0055] In other embodiments of the invention, the components of the PKS residing on any given polypeptide are derived from the same source, and are naturally contiguous in that source, but the intrapolypeptide linkers are changed to allow proper assembly across heterologous polypeptide junctions to form a functional PKS. For example, in one embodiment of the invention, the first polypeptide is the intact first polypeptide of the chalcomycin PKS, encoded by *chm*GI, and comprises the loading domain and first and second extender modules from the chalcomycin PKS together with the native C-terminal interpolypeptide linker region that directs interaction of the first polypeptide with the second polypeptide of the chalcomycin PKS. The second polypeptide comprises the N-terminal interpolypeptide linker and module 3 of the chalcomycin PKS, encoded by *chm*GII, but with the C-terminal interpolypeptide linker replaced by the C-terminal interpolypeptide linker from the second polypeptide of the spiramycin PKS, encoded by srmG2. This replaced C-terminal interpolypeptide linker directs the second polypeptide to interact with the third polypeptide, taken from the spiramycin PKS and encoded by the srmG3 gene. The remaining polypeptides are the third, fourth, and fifth polypeptides of the spiramycin PKS, encoded by srmG3, srmG4, and srmG5, respectively. In another embodiment of the invention, the first polypeptide comprises the loading domain and first, second and third extender modules from the chalcomycin PKS, together with a C-terminal interpolypeptide linker region derived from the C-terminus of the first polypeptide of the tylosin PKS. The remaining polypeptides are the third, fourth, and fifth polypeptides of the tylosin PKS. The use of the appropriate interpolypeptide linkers directs the proper assembly of the PKS, thereby improving the catalytic activity of the resulting hybrid PKS.

[0056] As noted above, the DNA compounds of the invention can be expressed in host cells for production of known and novel compounds. Preferred hosts include fungal systems such as yeast and procaryotic hosts, but single cell cultures of, for example, mammalian cells could also be used. A variety of methods for heterologous expression of PKS genes and host cells suitable for expression of these genes and production of polyketides are described, for example, in U.S. Patent Nos. 5,843,718 and 5,830,750; WO 01/31035, WO 01/27306, and WO 02/068613; and U.S. patent application nos. 10/087,451 (published as US2002000087451); 60/355,211; and 60/396,513 (corresponding to published application 20020045220).

[0057] Appropriate host cells for the expression of the hybrid PKS genes include those organisms capable of producing the needed precursors, such as malonyl-CoA, methylmalonyl-

CoA, ethylmalonyl-CoA, and methoxymalonyl-ACP, and having phosphopantotheinylation systems capable of activating the ACP domains of modular PKSs. See, for example, US Patent 6,579,695. However, as disclosed in U.S. Patent No. 6,033,883, a wide variety of hosts can be used, even though some hosts natively do not contain the appropriate post-translational mechanisms to activate the acyl carrier proteins of the synthases. Also see WO 97/13845 and WO 98/27203. The host cell may natively produce none, some, or all of the required polyketide precursors, and may be genetically engineered so as to produce the required polyketide precursors. Such hosts can be modified with the appropriate recombinant enzymes to effect these modifications. Suitable host cells include *Streptomyces, E. coli,* yeast, and other procaryotic hosts which use control sequences compatible with *Streptomyces spp.* Examples of suitable hosts that either natively produce modular polyketides or have been engineered so as to produce modular polyketides include but are not limited to actinomycetes such as *Streptomyces coelicolor, Streptomyces venezuelae, Streptomyces fradiae, Streptomyces ambofaciens,* and *Saccharopolyspora erythraea,* eubacteria such as *Escherichia coli,* myxobacteria such as *Myxococcus xanthus*, and yeasts such as *Saccharomyces cerevisiae.*

**[0058]** In one embodiment, any native modular PKS genes in the host cell have been deleted to produce a "clean host," as described in US Patent 5,672,491, incorporated herein by reference. The construction of the clean host *S. fradiae* K159-1, and the clean host *S. fradiae* K159-1/244-17a that produces methoxymalonyl-ACP are described below in Examples 2 and 3. Other organisms can be engineered using similar methods.

**[0059]** In some embodiments, the host cell expresses, or is engineered to express, a polyketide "tailoring" or "modifying" enzyme. Once a PKS product is released, it is subject to post-PKS tailoring reactions. These reactions are important for biological activity and for the diversity seen among 16-membered macrolides. Tailoring enzymes normally associated with polyketide biosynthesis include oxygenases, glycosyl- and methyltransferases, acyltransferases, halogenases, cyclases, aminotransferases, and hydroxylases. Tailoring enzymes for modification of a product of the chalcomycin PKS, a non-chalcomycin PKS, or a chimeric PKS, can be those normally associated with chalcomycin biosynthesis (including, but not limited to, proteins described in Table 2) or "heterologous" tailoring enzymes. As noted above, the P450 hydrolases encoded by the *chmHI, chmPI* and *chmPII* genes are of particular interest for production of polyketides having hydroxy groups well suited for subsequent chemical modification.

**[0060]** For purposes of the present invention, tailoring enzymes can be expressed in the organism in which they are naturally produced, or as recombinant proteins in heterologous hosts. For example, as shown in Examples 6 and 7, a hybrid PKSs having elements from the chalcomycin and spiramycin PKSs, or from the tylosin and chalcomycin PKSs were expressed in an engineered host derived from a tylosin producing strain of *S. fradiae* in which all or most of the post-PKS tailoring reactions of the tylosin biosynthetic pathway (see Baltz and Seno, 1988, "Genetics of *Streptomyces fradiae* and tylosin biosynthesis" *Annu Rev Microbiol.* 42:547-74) were expressed and which modified the polyketide product.

**[0061]** In some cases, the structure produced by the heterologous or hybrid PKS may be modified with different efficiencies by post-PKS tailoring enzymes from different sources. In such cases, post-PKS tailoring enzymes can be recruited from other pathways to obtain the desired compound. For example, as discussed in Example 6, a *chm*H gene has been used to modify the product of a chalcomycin-spiramycin hybrid PKS. Similarly, host cells can be selected, or engineered, for expression of a glycosylatation apparatus (discussed below), amide synthases, (see, for example, U.S. patent publication 20020045220 "Biosynthesis of Polyketide Synthase Substrates"). For example and not limitation, the host cell can contain the desosamine, megosamine, and/or mycarose biosynthetic genes, corresponding glycosyl transferase genes, and hydroxylase genes (e.g., picK, megK, eryK, megF, and/or eryF). Methods for glycosylating polyketides are generally known in the art and can be applied in accordance with the methods of the present invention; the glycosylation may be effected intracellularly by providing the appropriate glycosylation enzymes or may be effected in vitro using chemical synthetic means as described herein and in WO 98/49315, incorporated herein by reference. Glycosylation with desosamine, mycarose, and/or megosamine is effected in accordance with the methods of the invention in recombinant host cells provided by the invention. Alternatively and as noted, glycosylation may be effected intracellularly using endogenous or recombinantly produced intracellular glycosylases. In addition, synthetic chemical methods may be employed.

**[0062]** Alternatively, the aglycone compounds can be produced in the recombinant host cell, and the desired modification (e.g., glycosylation and hydroxylation) steps carried out *in vitro* (e.g., using purified enzymes, isolated from native sources or recombinantly produced) or *in vivo* in a converting cell different from the host cell (e.g., by supplying the converting cell with the aglycone).

**[0063]** It will be apparent to the reader that a variety of recombinant vectors can be utilized in the practice of aspects of the invention. As used herein, "vector" refers to polynucleotide elements that are used to introduce recombinant nucleic acid into cells for either expression or replication. Selection and use of such vehicles is routine in the art. An "expression vector" includes vectors capable of expressing DNAs that are operatively linked with regulatory sequences, such as promoter regions. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome.

**[0064]** The vectors used to perform the various operations to replace the enzymatic activity in the host PKS genes or to support mutations in these regions of the host PKS genes may be chosen to contain control sequences operably linked to the resulting coding sequences in a manner that expression of the coding sequences may be effected in an appropriate host. Suitable control sequences include those which function in eucaryotic and procaryotic host cells. If the cloning vectors employed to obtain PKS genes encoding derived PKS lack control sequences for expression operably linked to the encoding nucleotide sequences, the nucleotide sequences are inserted into appropriate expression vectors. This can be done individually, or using a pool of isolated encoding nucleotide sequences, which can be inserted into host vectors, the resulting vectors transformed or transfected into host cells, and the resulting cells plated out into individual colonies.

**[0065]** Suitable control sequences for single cell cultures of various types of organisms are well known in the art. Control systems for expression in yeast are widely available and are routinely used. Control elements include promoters, optionally containing operator sequences, and other elements depending on the nature of the host, such as ribosome binding sites. Particularly useful promoters for procaryotic hosts include those from PKS gene clusters which result in the production of polyketides as secondary metabolites, including those from Type I or aromatic (Type II) PKS gene clusters. Examples are *act* promoters, *tcm* promoters, spiramycin promoters, tylosin promoter (e.g., tylGIp, see Rodriguez et al., "Rapid engineering of polyketide overproduction by gene transfer to industrially optimized strains" *J Ind Microbiol Biotechnol.*

2003 Apr 16; and DeHoff et al., "Streptomyces fradiae tylactone synthase, starter module and modules 1-7, (tylG) gene, complete cds" Genbank Accession No. U78289), and other promoters. However, other bacterial promoters, such as those derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) and maltose, are also useful. Additional examples include promoters derived from biosynthetic enzymes such as for tryptophan (*trp*), the β-lactamase (*bla*), bacteriophage lambda PL, and T5. In addition, synthetic promoters, such as the *tac* promoter (U.S. Patent No. 4,551,433), can be used.

**[0066]** As noted, particularly useful control sequences are those which themselves, or with suitable regulatory systems, activate expression during transition from growth to stationary phase in the vegetative mycelium. The system contained in the plasmid identified as pCK7, i.e., the *actI*/*act*III promoter pair and the *act*II-ORF4 (an activator gene), is particularly preferred. Particularly preferred hosts are those which lack their own means for producing polyketides so that a cleaner result is obtained. Illustrative control sequences, vectors, and host cells of these types include the modified *S. coelicolor* CH999 and vectors described in PCT publication WO 96/40968 and similar strains of *S. lividans*. See U.S. Patent Nos. 5,672,491; 5,830,750, 5,843,718; and 6,177,262, each of which is incorporated herein by reference.

**[0067]** Other regulatory sequences may also be desirable which allow for regulation of expression of the PKS sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

**[0068]** Selectable markers can also be included in the recombinant expression vectors. A variety of markers are known which are useful in selecting for transformed cell lines and generally comprise a gene whose expression confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers include, for example, genes which confer antibiotic resistance or sensitivity to the plasmid. Alternatively, several polyketides are naturally colored, and this characteristic provides a built-in marker for screening cells successfully transformed by the present constructs.

**[0069]** The various PKS nucleotide sequences, or a mixture of such sequences, can be cloned into one or more recombinant vectors as individual cassettes, with separate control elements or

under the control of a single promoter. The PKS subunits or components can include flanking restriction sites to allow for the easy deletion and insertion of other PKS subunits so that hybrid or chimeric PKSs can be generated. The design of such restriction sites is known to those of skill in the art and can be accomplished using the techniques described above, such as site-directed mutagenesis and PCR. Methods for introducing the recombinant vectors of the present invention into suitable hosts are known to those of skill in the art and typically include the use of $CaCl_2$ or other agents, such as divalent cations, lipofection, DMSO, protoplast transformation, conjugation, and electroporation.

**[0070]** Expression vectors containing nucleotide sequences encoding a variety of PKS systems for the production of different polyketides can be transformed into the appropriate host cells to construct a polyketide library. In one approach, a mixture of such vectors is transformed into the selected host cells and the resulting cells plated into individual colonies and selected for successful transformants. Each individual colony has the ability to produce a particular PKS and ultimately a particular polyketide. Typically, there will be duplications in some of the colonies; the subset of the transformed colonies that contains a different PKS in each member colony can be considered the library. Alternatively, the expression vectors can be used individually to transform hosts, which transformed hosts are then assembled into a library. A variety of strategies might be devised to obtain a multiplicity of colonies each containing a PKS gene cluster derived from the naturally occurring host gene cluster so that each colony in the library produces a different PKS and ultimately a different polyketide. The number of different polyketides that are produced by the library is typically at least four, more typically at least ten, and preferably at least 20, more preferably at least 50, reflecting similar numbers of different altered PKS gene clusters and PKS gene products. The number of members in the library is arbitrarily chosen; however, the degrees of freedom outlined above with respect to the variation of starter, extender units, stereochemistry, oxidation state, and chain length is quite large. The polyketide producing colonies can be identified and isolated using known techniques and the produced polyketides further characterized. The polyketides produced by these colonies can be used collectively in a panel to represent a library or may be assessed individually for activity.

**[0071]** The libraries can thus be considered at four levels: (1) a multiplicity of colonies each with a different PKS encoding sequence encoding a different PKS cluster but all derived from a naturally occurring PKS cluster; (2) colonies which contain the proteins that are members of the

PKS produced by the coding sequences; (3) the polyketides produced; and (4) compounds derived from the polyketides. Of course, combination libraries can also be constructed wherein members of a library derived, for example, from the erythromycin PKS can be considered as a part of the same library as those derived from, for example, the rapamycin PKS cluster.

**[0072]** Colonies in the library are induced to produce the relevant synthases and thus to produce the relevant polyketides to obtain a library of candidate polyketides. The polyketides secreted into the media can be screened for binding to desired targets, such as receptors, signaling proteins, and the like. The supernatants *per se* can be used for screening, or partial or complete purification of the polyketides can first be effected. Typically, such screening methods involve detecting the binding of each member of the library to receptor or other target ligand. Binding can be detected either directly or through a competition assay. Means to screen such libraries for binding are well known in the art. Alternatively, individual polyketide members of the library can be tested against a desired target. In this event, screens wherein the biological response of the target is measured can be included.

**[0073]** Thus, the present invention provides recombinant DNA molecules and vectors comprising those recombinant DNA molecules that encode all or a portion of the chalcomycin PKS and/or chalcomycin modification enzymes and that, when transformed into a host cell and the host cell is cultured under conditions that lead to the expression of said chalcomycin PKS and/or modification enzymes, results in the production of polyketides including but not limited to chalcomycin and/or analogs or derivatives thereof in useful quantities. The present invention also provides recombinant host cells comprising those recombinant vectors.

**[0074]** Suitable culture conditions for production of polyketides using the cells of the invention will vary according to the host cell and the nature of the polyketide being produced, but will be know to those of skill in the art. See, for example, the examples below and WO 98/27203 "Production of Polyketides in Bacteria and Yeast" and WO 01/83803 "Overproduction Hosts for Biosynthesis of Polyketides."

**[0075]** The polyketide product produced by host cells of the invention can be recovered (*i.e.*, separated from the producing cells and at least partially purified) using routine techniques (e.g., extraction from broth followed by chromatography).

**[0076]** The compositions, cells and methods of the invention may be directed to the preparation of an individual polyketide or a number of polyketides. The polyketide may or may

not be novel, but the method of preparation permits a more convenient or alternative method of preparing it. It will be understood that the resulting polyketides may be further modified to convert them to other useful compounds. For example, an ester linkage may be added to produce a "pharmaceutically acceptable ester" (i.e., an ester that hydrolyzes under physiologically relevant conditions to produce a compound or a salt thereof). Illustrative examples of suitable ester groups include but are not limited to formates, acetates, propionates, butyrates, succinates, and ethylsuccinates.

**[0077]** The polyketide product produced by recombinant cells can be chemically modified in a variety of ways. For example , for example by addition of a protecting group, for example to produce prodrug forms. A variety of protecting groups are disclosed, for example, in T.H. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, New York (1999). Prodrugs are in general functional derivatives of the compounds that are readily convertible *in vivo* into the required compound. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs," H. Bundgaard ed., Elsevier, 1985.

**[0078]** Similarly, improvements in water solubility of a polyketide compound can be achieved by addition of groups containing solubilizing functionalities to the compound or by removal of hydrophobic groups from the compound, so as to decrease the lipophilicity of the compound. Typical groups containing solubilizing functionalities include, but are not limited to: 2-(dimethylaminoethyl)amino, piperidinyl, N-alkylpiperidinyl, hexahydropyranyl, furfuryl, tetrahydrofurfuryl, pyrrolidinyl, N-alkylpyrrolidinyl, piperazinylamino, N-alkylpiperazinyl, morpholinyl, N-alkylaziridinylmethyl, (1-azabicyclo[1.3.0]hex-1-yl)ethyl, 2-(N-methylpyrrolidin-2-yl)ethyl, 2-(4-imidazolyl)ethyl, 2-(1-methyl-4-imidazolyl)ethyl, 2-(1-methyl-5-imidazolyl)ethyl, 2-(4-pyridyl)ethyl, and 3-(4-morpholino)-1-propyl. In the case of geldanamycin analogs, solubilizing groups can be added by reaction with amines, which results in the displacement of the 17-methoxy group by the amine (see, Schnur et al., 1995, "Inhibition of the Oncogene Product $p185^{erbB-2}$ in Vitro and in Vivo by Geldanamycin and Dihydrogeldanamycin Derivatives,", *J. Med. Chem. 38*, 3806-3812; Schnur *et al.*, 1995 "*erbB-2* Oncogene Inhibition by Geldanamycin Derivatives: Synthesis, Mechanism of Action, and Structure-Activity relationships," *J. Med. Chem. 38*, 3813-3820; Schnur *et al.*, "Ansamycin Derivatives as Antioncogene and Anticancer Agents," U.S. Patent 5,932,655; all of which are

incorporated herein by reference). Typical amines containing solubilizing functionalities include 2-(dimethylamino)-ethylamine, 4-aminopiperidine, 4-amino-1-methylpiperidine, 4-aminohexahydropyran, furfurylamine, tetrahydrofurfurylamine, 3-(aminomethyl)-tetrahydrofuran, 2-(amino-methyl)pyrrolidine, 2-(aminomethyl)-1-methylpyrrolidine, 1-methylpiperazine, morpholine, 1-methyl-2(aminomethyl)aziridine, 1-(2-aminoethyl)-1-azabicyclo-[1.3.0]hexane, 1-(2-aminoethyl)piperazine, 4-(2-aminoethyl)morpholine, 1-(2-amino-ethyl)pyrrolidine, 2-(2-aminoethyl)pyridine, 2-fluoroethylamine, 2,2-difluoroethylamine, and the like.

**[0079]** In addition to post synthesis chemical or biosynthetic modifications, various polyketide forms or compositions can be produced, including but not limited to mixtures of polyketides, enantiomers, diastereomers, geometrical isomers, polymorphic crystalline forms and solvates, and combinations and mixtures thereof can be produced

**[0080]** Many other modifications of polyketides produced according to the invention will be apparent to those of skill, and can be accomplished using techniques of pharmaceutical chemistry.

**[0081]** Prior to use the PKS product (whether modified or not) can be formulated for storage, stability or administration. For example, the polyketide products can be formulated as a "pharmaceutically acceptable salt." Suitable pharmaceutically acceptable salts of compounds include acid addition salts which may, for example, be formed by mixing a solution of the compound with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, hydrobromic acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, benzoic acid, acetic acid, citric acid, tartaric acid, phosphoric acid, carbonic acid, or the like. Where the compounds carry one or more acidic moieties, pharmaceutically acceptable salts may be formed by treatment of a solution of the compound with a solution of a pharmaceutically acceptable base, such as lithium hydroxide, sodium hydroxide, potassium hydroxide, tetraalkylammonium hydroxide, lithium carbonate, sodium carbonate, potassium carbonate, ammonia, alkylamines, or the like.

**[0082]** Prior to administration to a mammal the PKS product will be formulated as a pharmaceutical composition according to methods well known in the art, e.g., combination with a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a medium that is used to prepare a desired dosage form of a compound. A pharmaceutically acceptable carrier can include one or more solvents, diluents, or other liquid vehicles; dispersion

or suspension aids; surface active agents; isotonic agents; thickening or emulsifying agents; preservatives; solid binders; lubricants; and the like. Remington's Pharmaceutical Sciences, Fifteenth Edition, E.W. Martin (Mack Publishing Co., Easton, PA, 1975) and Handbook of Pharmaceutical Excipients, Third Edition, A.H. Kibbe ed. (American Pharmaceutical Assoc. 2000), disclose various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof.

**[0083]** The composition may be administered in any suitable form such as solid, semisolid, or liquid form. See Pharmaceutical Dosage Forms and Drug Delivery Systems, 5th edition, Lippicott Williams & Wilkins (1991). In an embodiment, for illustration and not limitation, the polyketide is combined in admixture with an organic or inorganic carrier or excipient suitable for external, enteral, or parenteral application. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable carriers for tablets, pellets, capsules, suppositories, pessaries, solutions, emulsions, suspensions, and any other form suitable for use. The carriers that can be used include water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, talc, corn starch, keratin, colloidal silica, potato starch, urea, and other carriers suitable for use in manufacturing preparations, in solid, semi-solid, or liquified form. In addition, auxiliary stabilizing, thickening, and coloring agents and perfumes may be used.

**[0084]** It will be apparent from the forgoing that the invention provides may useful compositions and methods of using them. Without intending to limit its scope, in one aspect, the invention provides a recombinant DNA molecule that encodes a polypeptide, module or domain derived from a chalcomycin polyketide synthase (PKS) gene cluster. In an embodiment, the DNA molecule (or its complement) has substantial sequence identity to SEQ ID NO:1. In an embodiment, the DNA molecule hybridizes under stringent conditions to a nucleic acid having the sequence of SEQ ID NO:1 or its complement. In a related aspect, the invention provides a recombinant DNA molecule, comprising a sequence of at least about 200, optionally at least about 500, basepairs with a sequence identical or substantially identical to a protein encoding region of SEQ ID NO:1. In an embodiment, the DNA molecule encodes a polypeptide, module or domain derived from a chalcomycin polyketide synthase (PKS) gene cluster.

**[0085]** In one embodiment, the recombinant DNA molecule comprises a sequence encoding at least one module of a chalcomycin polyketide synthase. In an embodiment, the recombinant

DNA molecule encodes a chalcomycin polyketide synthase polypeptide selected from the group consisting of ChmGI, ChmGII, ChmGIII, ChmGIV, and ChmV.

**[0086]** In one aspect, the recombinant DNA molecule includes a coding sequence for a chalcomycin modifying enzyme, such as a chalcomycin P450 hydrolase enzyme selected from the group consisting of ChmHI, ChmPI, and ChmPII.

**[0087]** The invention also provides vector that comprise the recombinant DNA molecules of the invention. In an aspect, the invention provides a recombinant host cell comprising the vector. In a related aspect, the invention provides a recombinant host cell comprising a DNA molecule of the invention integrated into the cell chromosomal DNA.

**[0088]** Also provided is a chimeric PKS that comprises at least one domain of a chalcomycin PKS, and a cell containing such a chimeric PKS. In a related aspect, the invention provides a modified functional chalcomycin PKS that differs from the *S. bikiniensis* chalcomycin PKS by the inactivation of at least one domain of the chalcomycin PKS and/or addition of at least one domain of a non-chalcomycin PKS (for example, a loading domain, a thioesterase domain, an AT domain, a KS domain, an ACP domain, a KR domain, a DH domain, and an ER domain). The invention provides a cell comprising a modified functional PKS. The invention also provides a method to prepare an chalcomycin derivative which method comprises providing substrates including extender units to the cell.

**[0089]** In an aspect, the invention provides a recombinant expression system capable of producing a chalcomycin synthase domain in a host cell, said system comprising an encoding sequence for a chalcomycin polyketide synthase domain, and said encoding sequence being operably linked to control sequences effective in said cell to produce RNA that is translated into said domain, and a host cell modified to contain the recombinant expression system.

**[0090]** The invention provides an isolated polypeptide encoded by a recombinant chalcomycin polyketide synthase (PKS) gene, and a recombinant host cell containing or expressing such a polypeptide.

**[0091]** The invention also provides a recombinant *S. bikiniensis* cell in which a *chm*GI, *chm*GII, *chm*GIII, *chm*GIV, or *chm*V is disrupted so as to reduce or eliminate production of chalcomycin.

**[0092]** The invention also provides a recombinant DNA molecule encoding a first protein comprising one or more modules of a chalcomycin PKS and a second protein comprising one or

more modules of a tylosin PKS or spiramycin PKS, optionally one or more polypeptides of a chalcomycin PKS and one or more polypeptides of a tylosin PKS or spiramycin PKS. In a related aspect, the invention provides a recombinant host cell comprising a hybrid polyketide synthase comprising one or more modules of a chalcomycin PKS and one or more modules of a tylosin PKS or spiramycin PKS.

## EXAMPLES

**[0093]** The following examples are provided to illustrate, but are not intended to limit, the present invention.

Example 1 Isolation and Characterization of Chalcomycin PKS Cluster From *Streptomyces bikiniensis* NRRL 2737.

**[0094]** Growth of organism and extraction of genomic DNA. For genomic DNA extraction, a spore stock of *Streptomyces bikiniensis* NRRL 2737 was used to prepare a seed culture. Spores were stored as a suspension in 25% (v/v) glycerol at -80°C and used to inoculate 5 ml of unbuffered Trypticase Soy Broth (TSB) liquid media. The entire seed culture was transferred into 50 ml of the same growth medium in a 250 ml baffled Erlenmeyer flask and incubated with shaking for 24 h at 28°C. A 10 ml portion of the cell suspension was centrifuged (10,000 x *g*) and the resulting pellet was washed once with 10 ml buffer 1 (Tris, 50 mM, pH7.5; 20 mM EDTA). The pellet was suspended in 3.5 ml of buffer 1 containing 150 µg/ml RNase (Sigma-Aldrich) and 1 mg/ml lysozyme. After incubation of the mixture at 37°C for 30 min, the salt concentration was adjusted by adding 850 µl 5 M NaCl solution, then the mixture was extracted two times with phenol:chloroform:isoamylaclohol (25:24:1, vol/vol) with gentle agitation followed by centrifugation for 10 min at 3500 x g. After precipitation with 1 vol of isopropanol, the genomic DNA knot was spooled on a glass rod and redissolved in 200 µl of water. This method yielded about 0.5 mg total DNA. Standard agarose gel electrophoresis using 0.7% Seakem® LE-Agarose (BioWhiaker Molecular Applications, Rockland, ME) at a voltage of 50 mV overnight revealed that the sample contained mainly high molecular weight DNA of 50 kb or greater.

**[0095]** PKS Probe design. Five degenerate PCR primers were designed (degKS1F 5'-TTCGAY SCSGVSTTCTTCGSAT-3' [SEQ ID NO:44]; degKS2F 5'-GCSATGGAYCCSCARCARCGSVT-3' [SEQ ID NO:45]; degKS3F 5'-SSCTSGTSGCSMTSCAYCWSGC-3' [SEQ ID NO:46]; degKS5R 5'-GTSCCSGTSCCRTGSSCYTCSAC-3' [SEQ ID NO:47]; degKS7R 5'-ASRTGSGCRTTSGTSCCSSWSA-3' [SEQ ID NO:48]) based on conserved regions of ketosynthase (KS) domains of type I PKS genes and codon bias of high G+C organisms. The primers were used in the following combinations: degKS1F/degKS5R, degKS2F/degKS5R and degKS3F/degKS7R. The PCR conditions for the amplification of KS domains were as follows: A total reaction volume of 50µl contained 100 ng of *S. bikiniensis* total DNA, 200 pmol of each primer, 0.2mM dNTP, 10% DMSO and 2.5 U Taq DNA polymerase (Roche Applied Science, Indianapolis, In). Thirty-five cycles of PCR were performed using the following steps: denaturation (94°C; 40 sec); annealing (55°C; 30 sec); extension (72°C; 60 sec), 35 cycles. The resulting PCR reactions were subjected to electrophoresis on 1% agarose gels and the PCR products of approximately 700 bp were extracted from the gels using the gel extraction kit from Quiagen (Valencia, CA) according to manufacturer's protocol. The fragments were treated with *Pfu* DNA Polymerase (Stratagene, La Jolla, Ca) to remove the A overhangs and cloned into the plasmid vector pLitmus28 (New England Biolabs, Beverley, Ma) cut with *EcR*V. Thirty-two "amplimers" (the ca. 700 bp PCR-amplified segment) for each primer pair were sequenced using standard protocols. Of the 96 inserts sequenced, 81 were found to be KS amplimers. Employing the sequence comparison program ClustalW, 15 of the 81 KS amplimers were found to be unique and were compared with the 8 KS sequences of the related tylosin PKS cluster of *Streptomyces fradiae* using the program ClustalW. Each KS amplimer was thus assigned to a particular KS within the putative chalcomycin PKS cluster.

**[0096]** Genomic library preparation. Approximately 10 µg of genomic DNA was partially digested with *Sau*3A1 (1 hr incubation using dilutions of the enzyme) and the digested DNA was run on an agarose gel with DNA standards. One of the conditions used was found to have generated fragments of size 35-47 kb. The DNA from this digestion was ligated with pSuperKos plasmid, a derivative of pSuperCos (Stratagene) digested with *Afe*I and self-ligated to eliminate the *neo* marker, pre-linearized with *Bam*HI and *Xba*I and the ligation mixture was packaged using a Gigapack XIII (Stragene) *in vitro* packaging Kit and the mixture was

subsequently used for infection of *Escherichia coli* DH5α employing protocols supplied by the manufacturer . Approximately 2000 of *E.coli* transductants were probed by *in-situ* colony hybridization with DIG labeled Sb3/7-31 ($KS^q$), Sb1/5-75 (KS3) and and Sb1/5-78 (KS7). Plasmids from 15 colonies, which showed strong hybridization signals were isolated, digested with *Bam*HI and subjected to Southern blotting employing the $KS^q$ or KS7 amplimers as probes. Ten plasmids showed strong hybridization with one or both amplimers. The ends of the insert in each of the 10 plasmids were sequenced using convergent primers for each (T7 promoter and T3 promoter). Two cosmids, pKOS146.185.1 and pKOS146.185.10 were found to possess high homology at one end with a segment of the PKS from the tylosin biosynthesis cluster. These two plasmids also each gave rise to DNA fragments of ca. 1 kb and 1.2 kb after *Bam*HI-digestion.

**[0097]** Identification of chalcomycin biosynthetic gene cluster. Further verification that cosmids pKOS146.185.1 and pKOS146.185.10 contained the chalcomycin biosynthesis cluster was performed by PCR. Specific primer pairs were designed for the chalcomycin KSq (Sb3/7-31 forward 5'-CGTCAGCCTGATCCTCGCCGA-3' [SEQ ID NO:49]; reverse 5'-TCCAGGTGGCCGACGTTC GTC) [SEQ ID NO:50], KS3 (Sb1/5-75 forward 5'-AACGAGATCCCGCCGGG CCTC-3' [SEQ ID NO:51]; reverse 5'-ATCA CGCGTTGCTGGGCGAGG-3' [SEQ ID NO:52]) and KS7 (Sb1/5-78 forward 5'-GGACGTCTGCCGGAGG GTTCC-3'[SEQ ID NO:53]; reverse 5'-GGCCCGTTGGGCACGGACAGA-3'[SEQ ID NO:54]) amplimers and used in PCR reactions with each of the 8 KS amplimers using the following conditions: total reaction volume of 50μl contained 20-100 ng of plasmid DNA containing an amplimer, 100pmol of each primer, 0.2mM dNTP,10% DMSO and 2.5 U Taq DNA polymerase. Cycle steps were as follows: denaturation (94°C; 40 sec), annealing (55°C for KSq and KS3 specific primers, 65°C for KS5 specific primers; 30 sec), extension (72°C; 60 sec), 25 cycles. Each primer set was found to amplify its cognate amplimer exclusively, with the exception of the primer set for KS7, which was also seen to give a small amount of amplification of non-cognate amplimers. Each primer set was then used for PCR with cosmids pKOS146.185.1, pKOS146.185.10 and pKOS146.185.11 employing the same conditions as described above but using cosmid DNA in place of the plasmid-containing amplimer DNA. pKOS146.185.1 gave correctly sized amplimers with KSq and KS3 primers but not with KS7 specific primers, whereas pKOS 146-185.10 gave a correctly sized

amplimer with KS7 but not with KSq and KS3 specific primers, indicating that pKOS146.185.1 contained the 5' region of the chalcomycin PKS genes.

**[0098]** The sequence of the insert of pKOS146.185.1 corresponds to bases 1 to 48,595 of SEQ ID No.1 and the sequence of the insert of pKOS146.185.10 corresponds to bases 44,218 to 85,915 of SEQ ID No.1. Table 2 below provides open reading frame (ORF) boundaries corresponding to the nucleotide position in SEQ ID NO:1 (Table 3) of the chalcomycin PKS as well as the nucleotide sequences encoding enzymes involved in precursor synthesis and chalcomycin modification. In addition to the ORFs listed in Table 2, SEQ ID NO:1 includes additional open reading frames of genes encoding proteins or domains thereof that may be useful in the biosynthesis of chalcomycin and/or analogs thereof in certain host cells. The various open reading frames, module-coding sequences, and domain encoding sequences shown in Table 2 and the figures are sometimes referred to as "subsequences." Those of skill in the art will recognize, upon consideration of the sequence shown in Example 1, that the actual start locations of several of the genes could differ from the start locations shown in the table, for example due to the presence in-frame of codons utilizable by the initiator methionine tRNA in close proximity to the codon indicated as the start codon. The actual start codon can be confirmed by amino acid sequencing of the proteins expressed from the genes.

**Table 2- Chalcomycin PKS and modifying gene cluster ORFs of SEQ ID NO:1**

* "C" encoded by complement of SEQ ID NO:1

| ORF boundaries | Name | Proposed Function (homology) | Strand* | No. residues |
|---|---|---|---|---|
| 1 – 1009 | Orf1 | Orf1 complement<br>integral membrane protein (homolog of S. coelicolor SCF51A.28c) | C | 343 [SEQ ID NO: 2] |
| 1121 – 1963 | Orf 2 | Orf2 complement<br>(homolog of S. coelicolor SCF51A.29c) | C | 280 [SEQ ID NO: 3] |
| 3008 – 3889 | Orf3 | Orf 3 complement<br>Oxidoreductase (homolog of M.tuberculosis MLCL581.18c) | C | 293 [SEQ ID NO: 4] |
| 3991 – 5208 | chmCIV | 3,4-dehydratase, D-chalcose pathway (EryCIV homolog) | C | 405 [SEQ ID NO: 5] |
| 5339 – 6118 | chmMIII | D-chalcose O-methytransferase (SpnH homolog) | C | 259 [SEQ ID NO: 6] |
| 6239 – 7696 | chmCV | D-chalcose pathway (EryCV homolog) | C | 485 [SEQ ID NO: 7] |
| 7761 – 10271 | chmR | beta-glucosidase, extracellular reactivator of chalcomycin (OleR, DesR, EryBI homolog) | C | 836 [SEQ ID NO: 8] |
| 10306 – 11511 | chmPII | P450 C12, C13-epoxidase (MycG homolog) | C | 401 [SEQ ID NO: 9] |
| 11549 – 12772 | chmPI | P450 C8-hydroxylase (OleP homolog) | C | 407 [SEQ ID NO: 10] |
| 12762 – 13610 | chmI | TEII (homolog of TEII of tylosin cluster [the predicted product of ORF5 of GenBank accession # AF145042]) | C | 282 [SEQ ID NO: 11] |
| 13631 – 14602 | chmAII | TDP-glucose 4,6-dehydratase | C | 323 [SEQ ID NO: 12] |
| 14648 – 15562 | chmAI | TDP-glucose synthase | C | 305 [SEQ ID NO: 13] |
| 15869 – 16459 | chmJ | 3-epimerase; D-allose pathway (TylJ homolog) | C | 196 [SEQ ID NO: 14] |
| 16523 – 17290 | chmMII | D-mycinose 3' OH-MT (TylF homolog) | C | 255 [SEQ ID NO: 15] |
| 17551 – 18810 | chmHI | P450 C20-hydroxylase (TylHI homolog) | | 420 [SEQ ID NO: 16] |
| 18831 – 19052 | chmHII | Ferredoxin (TylHII homolog) | | 73 [SEQ ID NO: 17] |
| 18959 – 20029 | chmD | D-allose pathway 4-KR (TylD homolog) | | 326 [SEQ ID NO: 18] |
| 19049 – 20029 | chmD | Alternate N-terminus | | [SEQ ID NO: 19] |
| 20062 – 21273 | chmMI | D-mycinose pathway 2'OH-MT(TylE homolog) | | 403 [SEQ ID NO: 20] |
| 21329 – 22576 | chmN | D-allose glycosyltransferase (TylN homolog) | | 418 [SEQ ID NO: 21] |

| | | | | |
|---|---|---|---|---|
| 22653 – 23495 | chrB | Resistance determinant; 23S-rRNA N1-methyltransferase (TlrB homolog) | C | 280 [SEQ ID NO: 22] |
| 23622 – 36947 | chmGI | PKS, Modules 0-2 | | 4441 [SEQ ID NO: 23] |
| 23823-25046 | KSOq | PKS Ketosynthase 0q loading domain | | |
| 25353-26396 | AT0 | PKS Acyltransferase loading domain | | |
| 26499-26756 | ACP0 | PKS Acyl carrier protein loading domain | | |
| 26808-28079 | KS1 | PKS Ketosynthase 1 domain | | |
| 28386-29432 | AT1 | PKS Acyl transferase 1 domain | | |
| 30099-30794 | KR1 | PKS Ketoreductase 1 domain | | |
| 30966-31220 | ACP1 | PKS Acyl carrier protein domain | | |
| 31296-32567 | KS2 | PKS Ketosynthase 2 domain | | |
| 32889-33932 | AT2 | PKS Acyl transferase 2 domain | | |
| 33975-34574 | DH2 | PKS Dehydrogenase 2 domain | | |
| 35472-36257 | KR2 | PKS Ketoreductase 2 domain | | |
| 36402-36659 | ACP2 | PKS Acyl carrier protein 2 domain | | |
| 37041-42965 | chmGII | PKS, Module 3 | | 1974 [SEQ ID NO: 24] |
| 37143-38414 | KS3 | PKS Ketosynthase 3 domain | | |
| 38724-39869 | AT3 | PKS Acyl transferase 3 domain | | |
| 39903-40544 | DH3 | PKS Dehydrogenase 3 domain | | |
| 41442-42281 | KR3 | PKS Ketoreductase 3 domain | | |
| 42411-42668 | ACP3 | PKS Acyl carrier protein 3 domain | | |
| 43022–54388 | chmGIII | PKS, Modules 4 and 5 | | 3788 [SEQ ID NO: 25] |
| 43139-44422 | KS4 | PKS Ketosynthase 4 domain | | |
| 44750-45796 | AT4 | PKS Acyl transferase 4 domain | | |
| 46436-47248 | KR4 | PKS Ketoreductase 4 domain | | |
| 47318-47575 | ACP4 | PKS Acyl carrier protein 4 domain | | |
| 47651-48925 | KS5 | PKS Ketosynthase 5 domain | | |
| 49226-50272 | AT5 | PKS Acyl transferase 5 domain | | |
| 50309-51001 | DH5 | PKS Dehydrogenase 5 domain | | |
| 52085-52957 | ER5 | PKS Enoylreductase 5 domain | | |
| 52925-53728 | KR5 | PKS Ketoreductase 5 domain | | |
| 54439 - 59277 | chmGIV | PKS, Module 6 | | 1612 [SEQ ID NO: 26] |

| | | | | |
|---|---|---|---|---|
| 54544-55818 | KS6 | PKS Ketosynthase 6 domain | | |
| 56122-57168 | AT6 | PKS Acyl transferase 6 domain | | |
| 57844-58707 | KR6 | PKS Ketoreductase 6 domain | | |
| 58753-59019 | ACP6 | PKS Acyl carrier protein 6 domain | | |
| 59387 - 63439 | chmGV | PKS, Module 7 and TE | | 1350 [SEQ ID NO: 27] |
| 59489-60778 | KS7 | PKS Ketosynthase 7 domain | | |
| 61112-62209 | AT7 | PKS Acyl transferase 7 domain | | |
| 62276-62533 | ACP7 | PKS Acyl carrier protein 7 domain | | |
| 62549-63436 | TE | Thioesterase | | |
| 63522 - 64760 | ChmCII | NDP - hexose 3,4-isomerase; D-chalcose pathway component (EryCII homolog) | | 412 [SEQ ID NO: 28] |
| 64804 - 66081 | ChmCIII | Chalcose glycosyltransferase (EryCIII homolog) | | 425 [SEQ ID NO: 29] |
| 66194 - 66940 | ChmU | Post PKS Ketoreductase (SimJ2, NovJ homolog) | | 248 [SEQ ID NO: 30] |
| 67323 - 68471 | Orf4 | Permease homolog (SCF6.09 homolog) | C | 382 [SEQ ID NO: 31] |
| 68733 - 70196 | Orf5 | Membrane protein homolog (SC66T3.03 homolog) | C | 487 [SEQ ID NO: 32] |
| 70193 - 70888 | Orf6 | D-alanyl-D-alanine carboxypeptidase homolog (SCD6.17c homolog) | C | 231 [SEQ ID NO: 33] |
| 71382 – 72542 | Orf7 | Sensory histidine kinase homolog (SCE94.10 homolog) | C | 386 [SEQ ID NO: 34] |
| 72638 – 73324 | Orf8 | Two-component syst. response regulator homolog (SCE94.09 homolog) | C | 228 [SEQ ID NO: 35] |
| 73651 – 75081 | Orf9 | permease (xanthine/uracil permease type) (SC9G1.02, SC9G1.04 homolog) | C | 476 [SEQ ID NO: 36] |
| 75401 – 76117 | Orf10 | SC6A11.03c Homolog | C | [SEQ ID NO: 37] |
| 76537 – 78375 | Orf11 | Permease homolog (SC9G1.02, SC9G1.04 homolog) | | 612 [SEQ ID NO: 38] |
| 78521 – 79192 | Orf12 | MerR-family transcriptional regulator (SC1A4.06c homolog) | | 223 [SEQ ID NO: 39] |
| 79228 – 79983 | Orf13 | Type-II thioesterase (SanP homolog) | C | 251 [SEQ ID NO: 40] |
| 80489 – 81439 | Orf14 | Open reading frame | C | [SEQ ID NO: 41] |
| 81806 –82528 | Orf15 | Response regulator homolog (SCD49.02c homolog) | | 240 [SEQ ID NO: 42] |
| 82712 – 85912 | Orf16 | Open reading frame | | [SEQ ID NO: 43] |

[0099] Genes listed in Table 2 that encode proteins with post-PKS polyketide-modifying activities include: chmPI, chmPII, chmHI (P450 homologs), chmN, chmCIII (glycosyltransferases) and chmU (polyketide ketoreductase).

[0100] Genes listed in Table 2 that encode proteins predicted to participate in the biosynthesis of sugar residue subunits of chalcomycin or modification of sugar residues after their addition to the polyketide include: chmCIV, chmMIII, chmCV, chmAII, chmAI, chmJ, chmMII, chmD, chmMI, and chmCII. Of these, three are predicted to participate in D-chalcose residue biosynthesis (ChmCII, ChmCIV and ChmCV), two are predicted to participate in D-allose residue biosynthesis (ChmD and ChmJ) two are predicted to participate in conversion of the D-allose residue to D-mycinose residue after covalent linkage of the D-allose to the polyketide (ChmMI and ChmMII), two are predicted to provide precursors for both the allose and chalcose pathways (ChmAI and ChmAII), and one is predicted to O-methylate the chalcose residue (ChmMIII).

[0101] As noted above, the invention also provides inter-polypeptide linker sequences, which can be identified by the skilled reader (e.g., comprinsing the sequences between the N-terminus of the polypeptide and the beginning of the first KS domain; or between the C-terminue of the polypeptide and the beginning of the last ACP domain) and polynucleotides encoding such linkers.

Table 3

Chalcomyin PKS cluster from *Streptomyces bikiniensis* NRRL 2737 (SEQ ID N0:1)

```
   1 GGGGCCCGCC GGACGGGGCT GCCCGGCTCT CGGCGGTGCC CGGTGGGCCG GGTGCGGGCT
  61 CGCCCGCGGC GAGATGCTCC AGGACCTCCG CCAGTTCCCG GCAGGCGCGG CGTACCGAGC
 121 GGCGGGTCGC ACGCTGCTCC GTGATGACGG AGGCGAGAAG CAGGGCGGTC AGTGCGGCGG
 181 AACCGTTGAA CGCCTGGAGC TTGGCCATGA TCTCGACGTC CGAGAGGTGG AGGAACCCTC
 241 CCCGTCCGGC GTTCGCCTCG AAGGTGGCGA GCACGGAGGC GAAGAGCGCG CAGAGCATGC
 301 TTCCGGTGAG CTGGAAGCGG AGCGCCGCCC AGATCAGCAG GGGGAAGACG AGGAAGAGCA
 361 TGCCCACCGG GCTGAGCACG GCCATGGGCA TGAGGATCAG GGTCGCGAGA CCCAGCAGGG
 421 CCGCCTCCTT CCAGCGCCGT ACGCGGAACC GTCCGGCCGG CCCCGCGAGG ACGAGGAGGA
 481 GCGGGGCGAC GAGCAGCACC CCCATCGTGT CGCCCACCCA CCAGGCCAGC CAGACGGGCC
 541 AGAACTCGGT CGTGTCCAGG GAGCTCTTCG CCACCTGCAG TCCGACCCCG GCGGTCGCGC
 601 TGATCAGCAT GGCGCCGAAC CCGCCGAGGA AGACCAGGGA GAGTCCGTCC CGCAGCCGTG
 661 CCATGTCGAG CCGGAAGCCG GCCCGTGTCA GCAGCAGGAA GGCGCAGAGC GGCGCGACGG
 721 TGTTGCTGAC CACGGTGACC ACGGTGGTGG GCCCCGGCGT GGTGAGGGAG GCGATGACGA
 781 GGAAGGAGCC GAGGGCGATC CCGGGCCAGA CGCGCGCGCC GAGCAGCAGC AGGGCGGCGA
 841 CGGCGACGCC GGTGGGAGGC CAGATGGGGG TGACCACCAC GCCTTCGACG ACGAGGCGGC
 901 CCATCAGGCC GAGTCGTCCG GCCGCGTAGT AGAGCACCGC CACGGCCAGC GACATCAGCG
 961 CCGTCGCCGC GGGGGACCGG TACTGCCGAA TATCCAACAC GTCTGCCATC AGACACCGAC
```

1021 CGGGTTGCCG CGCCCCTGCA TGGCCGCCTG CGTGTCGGGG ACACGCCGCC CGGCCCGGAC
1081 CGGGCCGCGG GGAGGACCGG CCCGAGGCGG GTCCCCTCGC TCACTCCCCG GTCGCCGGCC
1141 GGGGCGGACC GCCGTCGTGG CCGACGACGA GGACGGCCGC GTCGTCCTCG TGCCCCACCG
1201 TGGCCGCCAG TCGCATGACG GCGGTGGCGA GCGCGTCGAC GTCCAGCCCG GCGACGGCCG
1261 TGATCCCGGC GAGTCTCGTC ACCCGGTCGA GACCCTCGTC GATGTGGAGC GAGGGCCCCT
1321 CCACGACCCC GTCGGTCAGG AGGACGAAGA CGCCGTCGGC GGTGAGCCGG TGGCGCGTGG
1381 CCGGGTAGTC GGCGCCGCGC AGCACGCCGA GAGGAGGCCC GCCCTCGCTG TCCTCGATGC
1441 CGGAGCGGCC GTCGGCGGTG GCCCAGATGT GGGGGATGTG GCCGGCCCGG GCGCATTCCA
1501 GGGTGCCGGC GGCGGGGTCG AGCCGCAGGA AGGTGCAGGT GGCGAAGAGG TCGGCGCCGA
1561 GGGAGACGAG CAGGTCGTTG GTGCGGCCGA GCAGCTCTCC CGGGTCCGCG GTGACGGAGG
1621 CCAGCGCGCG CAGTGCCACG CGGACCTGTC CCATGAAGGC GGCGGCCTCG ATGTTGTGTC
1681 CCTGGACGTC GCCGATGGAC ATGCCGATCC GCCCGCCAGG CAGGGGGAAG GCGTCGTACC
1741 AGTCGCCGCC GACGTTGAGC CCGTGGTTGG CGGGCTCGTA CCGGACGGCC AGCCGGGCAC
1801 CCGGAAGACT GGGCAGGTCC GAGGGCAGCA TGCCGCGCTG CAGGGCCACG GCGAGCTCGA
1861 CACGGGACCG CTGCGTCTCG GCCAGCTCCC GCGCCCTGGC GGTCAGCGAC CCGAGCCGGA
1921 CCAGAAGGTC CTCGCTGCTG TCGGCCGGGC GTCTGCGGAA CATCGATCAC TCCGACGTCA
1981 CGACAATCCT CGCATCACTC CGTCCCGTCT CCAGCACGCG GGACCACAGG GGACCACCCC
2041 GGTACGAACA GGTCCTTCCC ACTGTGCCCG GAGGGGGCGG GGTCCGCATC TCATCGGCGG
2101 GAGAGCGCGG TGGATCCCAG GGGGCCCGCT CAGGTCACCG AAAACGAGCA AACGTTCGAT
2161 AATGTGGTCG CGCCGGTCTG TGCGGCCGTT CAGCGTTCGA CGGTCACTGC GGCGGCCGCG
2221 ATGCCGTCGC GCACCAGCCA CCGGCCGGTG AACCCGCCCA GCTGCCGTCC GTCGACCGTC
2281 GGCCCGGGCA CGAGCAGCCG GGCCCTGAAA CGGCCGCCCG GCTGGAAGTC GACGGCCGCC
2341 TCCCCGAAGT CGAGCCGGCT CCGGGTGAGC GGGAACCACG CCTTGTAGAC GGCCTCCTTG
2401 GCGCAGAACA GCAGCCTGTC CCACGGGATG CCGGGCCGGG CCAGTGCCAG AGCGGCCAGC
2461 GGCCCGCGCT CATCCGCGGT CGTCACCGCC TCGAAGACGC CGTGCGGCAG CGGAAGGGCG
2521 GGCTCGGCGT CGATGCCCAG AGACGCCACG TCCGCCTCGG ACGCCACCGT CGCGGCGCGG
2581 TAGCCGGCGC AGTGGGTCAT GCTGCCGACG ACACCGGGCG GCCATCCCGG CGCACCCCGC
2641 TCACCGGGCA CGAGGGCCAC CGGCCCCCAC CCCAGACCGG CCAGCGCGCG TCGCGCGCAG
2701 AAGCGCACGG AGGTGAACTC CCTGCGTCGC TTGTCGACCG CGCGCCCGAT CGCCTTCTCC
2761 TCGTCGGCGA ACAGCCGCGC CTCGGGGTAC CGGTCGGCAT CCAGGACGTC GCCGAAGACG
2821 TCCACCGACA CCGCGGCGGG CGGCAGCAGT GCGCAGATCA GCCCGGCCCA CCGCGGCACC
2881 GACACGGCGG CGTCGGCCTC GGCACGGTCG TGCACGCACG CCGACGCCGC GGCGTCGGCG
2941 AGGGGGCCCG TGCGGCTGCC GTCCCCGGAC CGCCGCGCCG AGGACGCCGC CGGCGGGAAG
3001 CCGTCCCTCA CACGGGCACC GTGGCGTCGT CGTGCGTGCG TCCGAGCGCG TTCAGCCGGG
3061 CCAGCTGGCC ATCGGAGAGA GCGATGCCCG ACGCCGCGAC GTTCTCCCTC AGATGCGCGG
3121 GCGAGCTGGT GCCGGGGATG GGAATGACCG CCGGCGAGCG GTGCAGCAGC CACGCGAGCG
3181 CCACCTGACC GGCCGACACC TCCAGCTCGG TGGCGATGTC GGCCACCGGG CTCCCCCCGG
3241 CGGCGAGCGC ACCGCGGGCG ATCGGCAGCC AGGCGATGAA CGCGATCTCG TGCTTCTCGC
3301 AGTACTCGAC CACCTCGTCG TTGCGCCGGT CGGTCAGGTT GTACACGTTC TGCACGCTGG
3361 CCACGGTGAT GTGCTCACGG GCGGCCTCCA CTTCCCGGAC GGTGACCTTG GACAGCCCGA
3421 TGTGCCGGAT CTTCCCTTCG TCCTGCAGTT CCCCGAGCGC ACCGAACTGC TCGGCCGCCG
3481 GCACCTTCGG ATCGATCCGG TGCAGCTGGA AGAGGTCGAT GCGGTCGAGT CGCAGCCTGC
3541 GCAGGCTCAG CTCGGCCTGC TGGCGGAGGT ACTCCGGTCG GCCGCACGGC ACCCACTGGT
3601 CGGGACGGGG CCGGCACTGT CCGGCCTTCG TCGCGATCAC GAGGCCGTCG GGGTACGGGC
3661 GCAGTGCTTC GGCCAGCAGC TCCTCGTTGC TTCCCAGCCC GTAGGAATCG GCCGTGTCGA
3721 TGAAGGACAC ACCGAGGTCC ACGGCCAGCC GGGCCGTGCT GATCGCAGCC TCCCGGTCCT
3781 CCGGCGGGCC CCAGTACCCC GGACCGGTCA GCCGGAGGGC GCCGAAACCC AACCGGTGTA
3841 CCGAGAGGTC GCCTCCCAGA GGGAAGGTGG TCCTGCCGGG CTGTGCCATG CGTTCCTCCT
3901 GGACGACGTC CGTGCACTCG GGTGGGGCGC GTGGTGACCT CGGTGGGGGC GGGCCGATGC
3961 CGACCGTCCG AACACGGTGG ATCCCCCGGT TCAGGGAGCC GTCGTGGAGG GCACCCGCTC
4021 GTCGGCCCGT CGGGTCACCT CGTGTCCCCG CTCCAGAGTG AGCCGCACGA TGTCGCAGAC
4081 CCGACGGATG TCCTCGCGAG AGACGGTGGG GCCGGTGGGC AGGGCGAGGA CCTTCCGCGC
4141 GAGCCGTTCG GTGTGGGGCA GGGAGACCGG CCGCTCCGAG CGGTAGGGCT CCATCTCGTG
4201 GCATCCGGGG GAGAAGTACC GCTGGCACAT GATGTTCTCC GCCCTCAGCA CCTCGTCCAG
4261 CAGGTCCCGG TGGACCCCGG TCACCGCGGC GTCGACCTCC ACGACCAGAT AGTGGTAGTT
4321 GTTGCGCTCG GCACGGTCGA ACTCCATGAC CTTCAGCCCG GCGAGCCCGG CGAGTTCCGA
4381 CCGGTAGTCG TCGTGGTTGG CCTCGTTCCG GCGAACCGTC TCCTCGAAGG CGTCGAGCGA

4441 GGTGAGTCCC ATCGCGGCGG CGGCTTCGGT CATCTTCCCG TTGGTGCCGG TCTCCGTGGA
4501 CACCCGCCCC TGGGTGAATC CGAAGTTGTG CATCGCGCGG ACCCGCTGGG CCAGCCTCTC
4561 GTCATCGGTC ACCACCGCGC CGCCCTCGAA GGCGTTGACC ACCTTGGTGG CGTGGAAGCT
4621 GAACACCTCG GCTTGTCCGA AGCCGCCGAT CGGCTGCCCT TCCGACGTGC AGCCGAGTCC
4681 GTGCGCGGCA TCGTAGAAGA GCCTGAGACC GTGATCGGCC GCGACCTTCG CCAGCCGGTC
4741 GACCGCGCAG GGCCGACCCC ACAGATGGAC CGGCACGACC GCACTCGTAC GGGGGGTGAT
4801 CGCCGCCTCG ATCAGATCCG GGTCCAGGCA GTTGGTGGCC GGGTCGATGT CCACGAAGAC
4861 CGGGGTCAGA CCCAGCCAGC GGAACGCCTG GGCGGTCGCC GGGAAGGTCA GCGACGGCAT
4921 GATGACCTCG CCCGACAGAT CGGCGGCGCG GGCCAGCAAC TGGAGGGCGA CGGTCGCGTT
4981 GCAGGTCGAC ACGCAGTAGC GCACGCCGGC GAGTTCCGCC ACGCGCTGCT CGAACTCCCG
5041 GGCCAGCGGC CCGCCGTTGG TCAGCCACTG GTGGTCCAGC GCCCAGTTCA CACGGTCCAG
5101 GAACCGCGCT CGGTCGCCCA CGTTGGGCCG CCCCACGTGA AGTGGTTGCA GGAACGCCGA
5161 CGGGCCGCCG AACACGGCGA GATCACCGAG ATTGCGTTTC ATGGTCATAC CTCCCCGGTG
5221 CACGGGACGG TGCACCCGGC AGCAGCCGGC ACAGGACGTG GACGTACCGG AGGGACAGGG
5281 GCCGCAGCGC ACGACGGCGT CTCCGTCGTG CGTACGCGAA AGGGGCGCAC GGAACGGATC
5341 AGTGCTCGCG GCGCCAGTAG ACGCCCGTCA CGTCCACCGT CTCGATCGGC TCGTCGATGC
5401 CGTGTTCGCT CCGGTAGTCG TGGACGGCCT GCTTGCACGC CGGGATCAGG TAGTCGTCCA
5461 CGATCACGAA CCCGCCCACG GACAGCTTGG GGTAGAGGTT GACCAGCGCG TCCCTCGTCG
5521 ACTCGTACAG GTCGCCGTCC ACCCGCAGCA CCGCGAGCCG GTCGATCGGC GCGGTCGGCA
5581 GCGTGTCGGC GAACATGCCG GGCAGGAACC TGACCTGCTC GTCCAGCAGG CCGTAGCGGG
5641 CGAAGTTCTC CCTCACCTGC TCCTCGGAAC AGCTCAGTAC CCAGTTCAGG TGGTGGAACT
5701 CCATCGCCCG GTCGAGCGGG TGGCTGTCCT CGGACGTCAC CGGGACACCG GCGAACGAGT
5761 CGGCGAGCCA CACGGTGCGG TCCTCCACAC CGTGCGCCTT GAGGAGCGCG CGCATCAGGA
5821 TGCAGGCGCC GCCGCGCCAC ACGCCGGTCT CGATGAGATC ACCGGGAACA TCGTCCTGGA
5881 GGACCCTCTC CACGCACCTC TGGATGTTGT CCAGGCGTTG CAGGCCGATC ATGGTGTGCG
5941 CGACCGTCGG CACGTCCAGT CCTTCGGCCC GGTGGTCGGC GTCGAAGGTC CCGCGCTCGA
6001 CGTCCGGGCC CTCCGCCCCC GGACGCATGC GGTCCGCCAG TTCCTTGTCC AGGACGTTCA
6061 TCCCGACCGG GAAGGTCGGG TGATCCCCGT AGATGGTGTT CGACACGACG TTCTTCATGA
6121 GGTCCACGTA GAGCTCGCGG GTCTGCACGG TCGACTTACC TCCGGCTAGA TATCGAACAG
6181 AGAGAGAATG TGCGGGGCGA CGGCGTCCGC GGACGCGGTG GGAGCCGTCG CGGTCGGGTC
6241 ACCTCAGGAA GCCGCGCGCG TCCTCCCAGC CGGCCACGAC ATCGGCCTCC AGCTGGTTGA
6301 GCCGCGCCGC CACCACCTGG TCGAACCCGT CCATGAAGTA CTCGTCACCG GCGTGCGGCG
6361 CTATCAGACG GCCGTCGTCC ACGAACCGCT CGACGACCTC CGTCAGGGAG GTGCCCGGCC
6421 CCACCCGGCC CGCGACGTAC CGGTCCGCTC CGGTGAGATC CGGGAACCCC GCCTCCCGGT
6481 ACAGGTACAC GTCACCCAGC AGGTCCACCT GTACGGACAC CTGGGGATGC GCGGACCGGC
6541 GCATGGTTCC GGGCCTGATC CTGAGCAGCT CGGCGTCCGC GCCGGTCTTC AGGCTGTGCA
6601 ACGCGTAGCC GTAGTCGATG TTCAGCGTGG GGGTCCGCCG GCTCACCGCC TCCTCGAACG
6661 CGAGCAGCCC CTCCTGGAGT TCGGCGCGTT CGGCCTCGAA CAGCCTGCCG TCCTCCCGGC
6721 CGCTGTAGTC CTCGCGCACG TTCAGGAAGT CCACCGGCCG GTCCGGCGCG GCCTCGTTCA
6781 GGTCGGCGAT GAAGTCGACG AGGTCGAGCA GCCGGTGGAC GCGCCCGGGC AGCACGATGT
6841 AGCTGAGCCC GAGCCGGATC GGGCTCTCCC GCTCGGACCG CAGCTGCTGG AAGCGCCGCA
6901 GGTTGGCGCG GACCCTTCCG AAGGCGGCGC GCTTGCCCGT GGTCTCCTCG TACTCCTCGT
6961 CGTTCAAGCC GTACAGCGAG GTGCGCACCG CGTGCAGGTC CCACACCCCG GGCTGCCTGT
7021 CCAGCGTCCG CTCGGTGAGC GCGAACGAGT TCGTGTACAC GGTCGGCCGC AGCCCGCGAG
7081 CCGCCGCACG GCTGCTGAGC GCGCCCAGGC CCGGGTTGGT GAGGGGCTCC AGACCGCCCG
7141 AGAAGTACAT GGCGTACGGG TTGCCGGCCG GGATCTCGTC GATGACGGAC GCGAACATCG
7201 CGTTGCCGGA CTCCAGCGCC GACGGGTCGT ACCGCGCACC GGTCACCCGG ACGCAGAAGT
7261 GGCAGCGGAA CATGCAGCTC GGCCCCGGGT ACAGGCCCAC GACATAGGGG AAGGCCGGCT
7321 TGTGCGCGAG CGCCGCGTCG AAGACCCCCC GTCGCTCCAG CGGCAGCAGG GTGTTCTGCC
7381 AGTACTTGCC CGCCGGCCCG TTCTCCACGG CGTGGCGCAG CTCCGGGACA CGGCCGAAGA
7441 GGCCGAGGAG CCGGCCGAAG GAGGCCCGGT CCACGTCCAG CATCCGGCGT GCCTCCTCCA
7501 GGGGCGTGAA GGGGTGGTTC CCGTAGCGCT CGGCCAGCCG CACGAGCCGG CGCGCGACCG
7561 TCGGGCCCTC GTCGACACCG ACGCGGCCGT CGGAGACCAG GGCGCGGTGC ACCGTGTGGA
7621 CCGCTGCCGG CAGGTCGGCG CCGGGGCGGG CGCACAGGGC GACCGGATCG GCCGCGGTGG
7681 AGGCGTGCGA GGTCATCCTT CGTGTCCAAT CGTCTGCGTC CGGAGGGTCG GTCCGTTCCC
7741 CGGGAAACGC GGCGGGGCGG TCACCGGGAC GGCACCGTGA CGGATGTGGT CAGGGACGTC
7801 TCGGCCGCGG ACGGGCCCAC GTGTACGGCC CTCCTGCCGG TGCCCGGCTT CCACGTGCCG

```
 7861 GCGGCGGTGT CCCAGTAGCG CAGTTGCTGC TCGTCGACGG GGACGGTGAC CCGCCGCTGT
 7921 TCTCCCGGCC GGAGGGTGAC CTTGGTGAAT CCGGCGAGTT TGCGCAGCGC CTGGGGGGCC
 7981 TGGGTGTCCG GACTTGCCCC GAGGTAGACC TGGACGGTCT CCCTGCCCGC CCGGTCACCG
 8041 GTGTTGCGGA CGGTGACCGT GGCCTCCAGC CCCCGGGCGG TGCGCGCCAC GGACAGGTCG
 8101 CTGTAGGCGA AGGTGGTGTA CGACAGGCCG TACCCGAACG GGAAGAGCGG GGCCACGCCG
 8161 GTCCGGTCGT ACCACCGGTA GCCGACATCC AGTCCCTCGG AATAGGTCTC CTTGCCGTCG
 8221 ACACCCGGGT AGCGGTGCGG GTCACCGGCC ATGGGATGGC CGGTCTCGGT CCCCGGGAAG
 8281 GTCTGGGTCA GCCGGCCGCT GGGATTCGCG TCGCCGAACA GCAGGGCAGC GGTGGCCTCC
 8341 GCGCCCTCCT GCCCCGGGTA CCACATCTCC AGTACGGCGG CCGTCTCACG CAGCCAGGGC
 8401 ATGAGCACCG AGGAACCGGT GTTGAGGACG ACGATCGTGC GGGGGTTGAC CTTGGTGATC
 8461 GCCGCGATCA GCTCGTCCTG TCGGCCGGGC AGGGACAGCG AGGACCGGTC CACGCCCTCG
 8521 GCACTGTCGT CGTGGGCGAA GACGACGGCG GTGCGGGCCT CGGCCGCCGC CGCGACGGCG
 8581 CGGTCGAAGT CGTCCCGGGC AGCCTCGGGA GTGACCCAGC TCAGCTCCAC CGACAGCGGG
 8641 GTCTGTTCGA AGGCCCAGCC GTTCATGGTC ACCGCGTGGG TGCCGCGGGT CAGCCGCATC
 8701 GTGGTGGTGA CGGTGCCGAA CGCCTCGGTG CCGAGGACAG CGGACTGCCC CGCGATCTGC
 8761 AGGTTGGCGA CCCCGCCGAC GGCGGTGAAG GCGATCTTGT AGTCGCCGTC GGCGGGGACG
 8821 GTGAGCCGAC CCTCGTAGAA GACGCCCTGG CCGCTGGGAT CCAGTTGCTT CCCGCTCGTG
 8881 AAGGCGGGGG TGAGCGAGCT TTCGGGCACG GGCACGCCGA CCAGGTCCTC ACCGGGCTCG
 8941 TGGACGACCC GGGCCTTCTC GCCGGCGCGC CGTGCGAGGG CGTCGACGGG TGCGGTCGCC
 9001 CGGTCGGGTA TGACGTGCGC GCTGCCGTTG CCGGTGACCT TGGGGTGCTT GGCGCTGTTG
 9061 CCGATGACGG CGATGTCCGT GGCGTCCTCA CCGGTCAGGG GCAGCGTCCG GCGCTCGTTG
 9121 CGCAGCAGCA CCCCGCCGTT CTCGGCGATG GTGCGGGCCG CCGCGCGGCC GGCCTCGGGA
 9181 TCACGCTGCG GGCGCTCGGT CGCCTTGCCG TCGAGCAGGC CGAAGCGCTC CATCTGGCCG
 9241 AGGATGCGGA CGACGGACCG ATCGAGCGTC GCCTCGGGGA CGCTGCCGTC CCGCACGGCG
 9301 GCCCTGAGCG CGGAGGAGAA GTACTTCGAT TCCGGCACCG GCTGCCCCAG GGTCAGCTCG
 9361 ACGCCCAGTT CCTGGTCGAG GCCCCTGGTG ATGTCGCCGG TGGCGTGGGT CGCCAGCCAG
 9421 TCGGACACCA CCCAGCCGCG GAAGTCCCAC TGTTCGCGCA GCACCTCCTG CAACAGGTGC
 9481 TCGTTCCCGC ATGCGTGCGC GCCGTTGACC TTGTTGTACG AGCACATCAC CGAGGCGGCG
 9541 CCCGCCCGCA CGGCGCTGCG GAAGGCCGGC AGCTCCACCT CCTGGAGCGT CTGCTCGTCG
 9601 ACCACGGCGT CGATGGTCTC CCGCTGGTAC TCCTGGTTGT TCGCGGCGAA GTGCTTGACC
 9661 GTGGCCATCA GGCCCTGGTT CTGGATGCCG CGGACCTCGT GGGCGGCCAT CCGGGACGAC
 9721 AGCAGAGGAT CCTCGCTGTA GGTCTCGAAG TTCCGCCCCG CGTGCGGCAC CCGGATGACG
 9781 TTGGTCATGG GACCGAGGAC GATGTCCTGT CCGAGGGCCC GTCCCTCCCG GCCCAGGACG
 9841 GTGCCGTACT CCTCGGCGAG GCGTTCGTCG AAGGTGGCGG CGAGCGCCAC GGGGGTGGGC
 9901 ATGGCGGTGG CGGTACCGCC GAGCAGACGG ATGCCGACCG GGCCGTCGGC GGTGCGCAGC
 9961 TCGGGGATGC CGAGCCGGGG CACGCCCGGC AGATAGCCGA TGCCGGTCAT GGTCGGCCCG
10021 CCCACGGGCC CGGTGGTCCA GTGGACGAAC GAGATCTTCT CGTCGAGGGT CATCTTCGCC
10081 ACCAGCCCGC GCACCCGCGC CGTCCCGGGC TCGGCGGCCG GAGCACCGGC GGCGGAGGGG
10141 GCGGTGAGCA GACCGCCGAC GCACAGGGCC GCGAGCGCGG ATCCGACGGT GCGCCGGATG
10201 CGGCGACCCG TCCTCGTCGT GCCGAACAGC ATGCTGACGG ACGTCCTTTC TGCCGAGGTT
10261 GCCGTCCTCA TCGGGGCGGG AACGTTTCTG TCCGTGCCGC CATGATCACC AGCCCACGAG
10321 CATCGTGCGC GGGCCCCGCA CCACCATCTC CGTCTTCCAC TCGACGGGCT GGGCGAGGTG
10381 CAGCCCGGGG AGTTCGTCGA GCAGGGTCCG CAGCGCCTCC TGGAGCTCCA GCCGCGCGAG
10441 GGACGCGCCG AGGCAGTGAT GCGGACCGTG TCCGTACCCC AGGTGCCGGC CGGCGTCGTC
10501 GCGGGTGATG TCGAGCACGC CGGGCGAGCG GAAGCGCAGG GCGTCCCGGT TGGCGGCGTT
10561 CATCTGGACC AGCACCGGAT CCCCCGCCCG CACCAGCGTC CCGCCCACCT CGACGTCCTC
10621 GGTGGCGTAG CGCGGGAATC CCGCCTGGCT GCCGAGCGGA ACGAACCGCG TCAGCTCTTC
10681 CACCGCGTTG TCCAGCAGGT CAGGACGGTC CCGGAGGAGG GCCAGCTGGT CCGGCTGTTC
10741 GAGCAGGACC AGGACGAAGT TGCTGATCTG GCTGGCCGTG GTCTCGTGCC CGGCGAACAG
10801 GAGGAAGACG ATCAGGTCGA CCAACTCCTC CTGCGACAGC CGGCCCTGGG CGTCACGGGC
10861 CTCGACGAGC GCCGAGACGA GATCGTCTCG CGGCGCGGCG CGGCGGGCCG TGATCAGGTC
10921 CGCCAGATAG CCGGTCAGCT CGCCGGCCAG CCGCACATGG TCCTCGGCGG TGAGCGAGCT
10981 GGTGGACATC GCTATCTCGC ACCAGCCGCG GAACAGGTCA CGGTCCTCCT CGGGCACGCC
11041 CAGCAGGCCG CAGATGACCG CCACGGGAAG GGGGACGGCG TAGTGGTCCA CGAGGTCGAC
11101 GGGCGATCCG AGCGCCGTCA TGTCCCGGAG CAGCGACGCC GTCATCCTTC GGACGTGCGG
11161 CCGCATGGCC TCCACGCGGC GCGGGGTGAA CGCCTTGGTG ACCAGCCCGC GCAGCCTGGT
11221 GTGGTCCGGC GCGTCCATGC CGATGATGCC GTTGGGCGTC CGCAGCTCCC GCATGCGCGG
```

```
11281 CGCGTCGTTC TCCGCCTCCG CGGCGTGGCT GAACCGCCGG TCGCCGAGGA CGAAACGCGC
11341 GTCGTCGTAA CGGGAGACGA GCCAGCCCGG CTCCCCGTAC GGCATGTGGA CCCAGAGGAG
11401 TCCTTCGCTC TTCCGCGCCT GCTCGTACGC CTCGTCCAGG TCCAGTCCCT CCGGGCGGCC
11461 GAAGGGATAG GAGACGGGTG CGGTCTTCGT GCTGGTCAAG GGGAGCCCCA TATCGCGTCG
11521 TTCGGGTGCT TAATCGTCGT GGGCCGGATC ATGCGGCCGA GCCGCCCCAG GTGACGGGGA
11581 CCGCCTCGGG GGTGCGCAGG AGGGAGCCGG CCCGCCAGCG AAGCTCACTC GCGGGGACGG
11641 ACAGCCGGAG CTCCGGCAGG CGCTCGACCA GGGTGCTCAG CACCACCTGC AGTTCGAGCC
11701 GGGCCAGGGG GGCGCCCATG CAGTAGTGGG CGCCGTGACC GAAGCCGAGG TGCGGGTTCT
11761 GGGCACGGGC CAGGTCGAGG GAGGCGGCAC CGGGAAACGC CGTGTCGTCG AGGTTCGCGG
11821 ACGCGATCGA GGTGATGACC GCCTCGCCCG CGCGGATGAG CGTGCCGCCG ATCTCGACGT
11881 CCTCCAAGGC GATGCGGGCG AACCCGTCCA GGGTGCTCAG CGGGGTGAAG CGCAGCAGCT
11941 CCTCGACGGC GGAGGGAACC AGGTCAGGTT CGGTCACCAG GCGCTCGTAG AGGCGGCGGT
12001 CGTCGAGCAG GAGGAGGACG AAATTGCCGA TCTGGGTGGC GGTCGTCTCG TATCCGCTGA
12061 TCAGCAGGCC GGAGCCCATC ATGAGGAGCT CGGCCTCGGA GAGCCGGTCG CCTTCGTCAC
12121 GGGCCTGGAC CATGGTGCTG AGGAAGTCGT CGGTGGGCCG GGCGCGGCGA TCGGCCACGA
12181 GGTCGGCCAT GTAGGCGGAG AAGTCCTCGG TCGCCCGCTG CACTTCGGCG GGCCCCAGGG
12241 AGGAGGACAC CAGCGCCTCG GAGAAGTCCC GGAAGAGGTG GCGGTCGTCG TAGGAGACGC
12301 CCAGCAGTCC GCAGATCACG GAGATCGACA CGGGCAGCGC CAGGTCTTCC ATGACCTCGC
12361 CGGACGGCTC GTCGCGCGCG ACCATGCCGT CGAGCAGTTC ATCGGTGAAC CGTGAGACCC
12421 GCGGGCGCAG TTCCTCCACC CGGCGGGCGG TGAACGCCTT CCCGGCGAGC CGCCGGAGCC
12481 TGGTGTGTCC GGGCGGGTCC ATGGAGATGA GACCGCCGGC GGGCAGCGGG TACTCGGTGG
12541 GCCGGGGCAC GTCACGTCCG GCCCCGGCCT CCATGCTGAA CCGCGGGTCT CCCAGGACCG
12601 CCCTCACGTC GGCGTGTCGG GTCAGCAGCC AGGCCTCACC GCCGTACGGC ATGCGGATCC
12661 GGGACACGGG CTCGTGCTCC CGCAGACGGC TGTAGAGCGG ATCCAGCGCG ATGCCCTCGG
12721 GCGGGGAGAA GGGGTACGGG CGAGTCCGGT GGACCGGACA GCTAGACGTC ATGGACACTC
12781 TCTCGGTCGT ACGGGGCGGC GGCAGAGCTC GAAGGGTGCG GGCGGCCGAC GGCGAGCCCG
12841 GTCGCCGTCC GGATCGTCCG GCACACCGCT TCGGCCTGCT CGGTGAGGTA GAAGTGGCCT
12901 CCGGGGAAGG AACGGAACGC CGTCTCCCCG GTGGTCAGCC CGTGCCAGGC CAGGGCTCCG
12961 TCGGTGGGGA CGTGCGGATC CGCGCTTCCC GTCAGCACGG TGATCGGGCA GGCGAGCGGA
13021 GCTCCCGGAC GCCACGTGTA GGTGGCGGCG GCCCGGTAGT CGTTGCGGAT CGCCGGCAGG
13081 GCCATCCGCA GGACCTCCTC GTCGGCCAGC ACCCGGGGGT CGGTGCCTTG CAGTTCGGCC
13141 AGCTTGGCGA CCAGCCGGTC GTCGCTCAGC AGGTGCGCGG TGGTCCGGTG GGCGACGGTC
13201 GGGGCGACGC GGCCGGAGAC GAACAGATGC GCCAGCCTGC TCTCCGGCGC CTGTTCCGCG
13261 AGCCGGCGGG CGGTCTCGAA GGCCACCAGC GACCCCAGGC TGTGACCGAA GAGCGCCACC
13321 GGCCGGTCCA GCCAGGGGCC GAGCGCTCCG GCGACGTGCG TCACCAGAGC GTCGACGGAG
13381 TCCACGAACG GCTCCAGCCG CCGGTCCTGC CGCCCGGGAT ACTGGACGGC GAGCACCTCG
13441 ACCCGGTCCG GCAGCAGACA GGTGAAGGGG AGGAAGGCGC TGGCGCTGCC GCCGGCGTGC
13501 GGAAGGCACA CGAGCCGGAG CGCGGGGTCG GCGACGGGCC GGTAGCGGCG CAGCCAGAGG
13561 TCGCCGGCGT TGGTGGAGCC GGGCGCCGTC GTGGCGGGCC CCTCGTTCAT GCGTGATCCG
13621 CTTCCGGACG TCACGGTGTC AGCGCCTTCC ACCACGTGCC GTTGTCCCGG TACCAGTCGA
13681 CGGTCTCCGC GAGCCCGCGA TCGAAGGCGA CGCGGGGAGC CCATCCCAGC TCCTCGGTGA
13741 TCTTCCGGGT GTCCACGCAG TAGCGACGGT CGTGCGCGGC CCGGTCCGGC ACCCGGTCCA
13801 CCACGGACCA GTCGGCACCG AAGACGCCGA GAAGACGACG GGTGAGGTCG ACGTTGGACA
13861 GTTCCGTCCC GCCGCCGATG TTGTACACCT CTCCGGCCCG CCCCCGGGCG GCGACCATGG
13921 CGATGCCCCG GCAGTGGTCG TCCACGTGCA GCCAGTCGCG CCGGTGGAGG CCGTCCCCGT
13981 ACAGGGGAAG GCGCAGGCCC TCCAGCAGAT GGGTCACGAA GAGCGGGACG ACCTTCTCGG
14041 GGTGCTGGTG GGGGCCGTAG TTGTTGGAGC AGCGCGTCAC CCGGACGTCG AGGCCGTGGG
14101 TACGGTGGAA GGCGAGCGCC AGGAGGTCGG AGGAGGCCTT GGACGCCGCG TAGGGGGAGT
14161 TCGGGTCCAG CGGATCGGCC TCGGTGGAGG AGCCCTCGGG GATCGATCCG TACACCTCGT
14221 CGGTGGAGAC GTGGACGAAG CGGCTGACGC CCGCTTCGAG CGCCGACCGC AGCAGGGTCT
14281 GCGTCCCCAG CACGTTGGTG GTGACGAAGG CGGCGGAGTC CGTGATCGAG CGGTCCACGT
14341 GCGACTCGGC GGCGAAGTGC ACCACCAGGT CGGTGCCGCG CAGGGCCTGG GCCACCGTGG
14401 GCGGGTCGCA GATGTCGCCG TGCAGGAAGG TGTGACGGGG GTGGCCGGCG ACCTCGGCGA
14461 GGTTCTCGGT GTTCCCGGCG TACGTGAGCT TGTCCAGCGA GAGGACGTGG GCGCCGGCCA
14521 GCTCCGGATA GGAGCCCGAC AGGAGCTGTC TGACGAAGTG CGAGCCGATG AAGCCCGCTG
14581 CCCCGGTCAC CAGGACGCGC ATCCTTGATC CGCCTTTCTG CCGTGCTGTG TGGGGGAGGT
14641 GTGGTCAACC GGTGCGCGTC GCCCCGAGTC CCGAGGCGAC CTCCATGAGG TAGTCCCCGT
```

```
14701 AGCTGGAGGC GCTCAGCTCC TCACCCAGCC GGTAACAGGT GTCCGCGTCG ATGAAGCCCA
14761 TGCGCAGCGC GATCTCCTCG AGACAGGCGA TGCGTACGCC CTGGCGCTGC TCGAGGAGCT
14821 GGACGTACTG ACCCGCCTGG AGCAGGGAGT CGTGGGTTCC CATGTCGAGC CAGGCGAATC
14881 CACGTCCGAG CTGGGTCAGC CGGGCGCGGC CCTGCTCCAG ATAGGACAGG TTGATGTCGG
14941 TGATCTCCAA CTCGCCTCGG GCTGAGGGCT TGAGGTCCTT CGCCAGCTCC ACCACGTCGT
15001 TGTCGTAGAA GTACAGCCCG GTGACGGCGA GGTTGGAGCG AGGACGGCTC GGCTTCTCCT
15061 CCAGGGACAA CAGGTGCCCC TGCTCGTCGA TCTCGGCGAC GCCGTAGCGC TCGGGCGACT
15121 TGGACGGATA GCCGAACAGT TCACATCCGT CGAGACGTCG CAGGGAGTGC TTCAGGACGG
15181 TGGAGAATCC GGGGCCGTGG AAGACGTTGT CGCCCAGAAT GAGCGCCACC CGGTCGTTCC
15241 CGATGTGCTT GTCGCCGACC AGGAACGCGT CGGCGACACC GCGCGGCTCG TCCTGGACCG
15301 CGTAGTCGAG CTGGATACCG AGGCGCGAGC CGTCGCCGAG CATGACCTGG AACGTCTCCA
15361 CGTGCTGGTG CGAGGAGATG ATGAGGATGT CCTGGATTCC CGCCAGCATC AGCACCGACA
15421 GCGGATAATA GATCATGGGT TTGTCATAAA CGGGCAACTG CTGTTTGGAA AGCGCACCGG
15481 TCAGCGGCTG CAGTCGAGTG GCGCTTCCCC CGGCGAGGAT TATCCCCCTC ACTCCGGGGC
15541 GTTTCAGCGG TGTCTCGAAC ACGGTTGGTC CTCCGTGGTC ACATGGCCGA TATGGGGGGT
15601 GAAGACACTG TCCTGAGAGG CCGGCGGACC GGCTGTCGCC TCGCGGACAC AGCGGCTTAA
15661 TGCATTCACC CCGCCCCGGG ACCGTCATCC GAGAAGAAGG AATGCGGTGT CGTGGGAACC
15721 GACGTCCAGG AGTTCCTTTC GGGCCGCGGA ACGGCGGCGC GGAGATTCTG AACCGCGGGG
15781 GATTCCAGGG CGGTGGCAGG GAAGGGAACC ACCGCCGCGC CATCTCTCCC GGAACGTTCC
15841 GCAAGCGGCG GGCCGTGCTT CGGACGGCCT ATCTCTGCGC CTGTTGCTGT TCCTGCCAGG
15901 CCTGATAGGT CGGCAGCAGA CCGAGCCGTT CGGCCGTCGC GAGGGTCGGG GCGTTCTCGT
15961 CCCTGTCGGA GAGCAGCGGC TCGATGTCGT CCGGCCACGC GATTCCGAGG TCGGGGTCGA
16021 GCGGATTGAC GGAGTGCTCG CGTGCGGGAG CGTATCCGGA GGAGCAGAGG TAGACGAGCG
16081 TGGCGTCGTC GGTGAGGGAG AGGAAGGCAC GGCCCAGTCC CGCGGTCAGA TAGACGGCGG
16141 TGTTGCGTTC CGCGTCCATG GGCACGATCT CCCAGCGCCC GAAGGTCGGC GAACCGATGC
16201 GGACGTCCAC GACGACGTCG AGGCCGGCTC CCCGCACGCA CACGCTGTAC TTGGCCTGAC
16261 CTGGCGGGAT CTCGGTGTAG TGGATCCCGC GCAGCGCGCC GCGGTGCGAC ACCGCGACAT
16321 TGACCTGGGC CACCGGGAAG TCATGGCCGA ACGCCTGACG GAAGCTCTCG CCCCGGAACC
16381 ACTCGTGGGA CCTCCCGCGG TGGTCGGAGT GGATGACGGG TTCCTGCGAC CAGGCCCCCT
16441 CGATGCTGAG TGGATGCATC GCGCCTTCTC CTTCGGACCG ATGGGTGGGG TGCGGGGCGG
16501 GCCGGGGGCA CGGCCGAGCC GGTCAGCTGG AGCGTCTCCA GTAGGAACCC TGGCGATCGA
16561 TCTGGTGGAT CTCGTCCGTG ATGCCGTGCT CGTCCCGGAA CTCGTGGACG GCCTGCCGGC
16621 ACGCCGGAAT GCAGTAGTCG TCGACGATGA CGTACCCGCC GTCCGACACC TTGTGGTAGA
16681 GGTTGGTGAG AACCTCCCTG GTGGCTGCGT ACGAGTCCCC GTCGAGCCTC AGCACCGCGA
16741 GCCTCTCGAT GGGCGCGGTG GGCATCGTGT CCTTGAACCA GCCCGGGAGG AAGCGGACCT
16801 GGTCGTCCAG GAGTCCGTAG CGCGCGAAGT TCCCCTTCAC GGTCTCCACG TCGACCGGGA
16861 TGCTGAGGAC GTCGTTGTAC TGGCCGAGGT CGATGTCGAC GTCCAGCTGG TGGTCGTCCT
16921 CGGTGGTCTT GGGGAAACCC TGGAAGGAGT CCGCGACCCA CACCTTCCGG TCCCGCACGC
16981 CGTGCGCCCG GAAGACGCCG CGGGCGAAGA TGCAGGCCCC GCCCCGCCAG ACCCCGGTCT
17041 CCGCGAAGTC CCCCGGCACG CCGTCGCGCA GCACGTCCTC CAGGCACTTC TGCAGGTTGT
17101 CGAGCCGCTT GAGACCGACC ATCGAGTGCG CCACGCGCGG AAAGTCCTCA CCCACCGAGC
17161 GCAGTTCCGC GGAATACGAG CTGCTGGTGA TGAGGCCGGC GACATTGGTC TGGTCCTCGT
17221 AAATCATGTT CGTCACGACC TTCTTCAGAA GGTCGAGATA CAGGTCCGCT TCGGCAGCTA
17281 TGACAGTCAT TTTCCTCACT TACGGGTAGC AGTGCCCAGC GGGCGGCTCG TTCAGGACGG
17341 GGGCCGCCGG GGCTGAATTC CCTGTGTCCA CACAGATGAG GTGGATGAGG TGGATGAGGT
17401 AGCCATCTAA CCCCAGTGAT CAGATTCGGG CAAGGGTCGA AAACGAGCCA CGTCTTATGT
17461 CGATCCTGTC CGGAAGCGAG GGGCATATGG TGCAGTGGCG ACTGCGGCCG ATCTGGCTGA
17521 TCCTTGCTGC GGCGCTGACC GTGTGCTTGC ATGTTGGACG TAGATCACCT TCTCCCGATT
17581 GCATTCAGGG TGAGGAAATC CATGAAATCT TCAAAAGTCG TTCACAGCCG TCCTGCGGAA
17641 GCGGGCGTCG CATGGCCCGT CGCGCGAACC TGCCCCTTTA CGCTCCCTGA TCAGTACGCA
17701 GAGAAGCGCA AGAACGAGCC CATATGCCGG GCTCAGGTCT GGGACGACTC CAGAACCTGG
17761 CTCATCACCA AGCACGAGCA CGTACGAGCC CTTCTCGCCG ACCCCCGGGT CACCGTCGAC
17821 CCGGCCAAGC TGCCCAGGCT CTCCCCCTCC GACGGCGACG GCGGCGGCTT CCGGTCCCTG
17881 CTGACCATGG ACCCCCCGGA CCACAACGCC CTCCGCCGCA TGCTCATATC CGAGTTCAGC
17941 GTGCACCGGG TCCGGGAGAT GCGCCCGGGC ATCGAGCGCA CCGTGCACGG GCTGCTGGAC
18001 GGGATCCTCG AACGGCGGGG GCCGGTGGAC CTGGTGGCCG AACTCGCGCT GCCGATGTCC
18061 ACCCTGGTGA TCTGTCAGCT CCTCGGAGTG CCCTACGAAG ACCGCGAGTT CTTCCAGGAA
```

18121 CGCAGCGAAC AGGCCACCCG GGTGGGCGGG AGCCAGGAAT CGCTGACCGC GCTCCTGGAA
18181 CTACGGGACT ACCTGGACCG GTTGGTCACC GCGAAGATCG AGACGCCGGG TGACGACCTG
18241 CTGTGCCGGC TCATCGCCAG TCGACTGCAC ACCGGTGAGA TGCGACACGC CGAGATCGTG
18301 GACAACGCCG TGCTGCTGCT CGCCGCCGGC CACGAGACCA GTGCCGCCAT GGTGGCACTG
18361 GGCATCCTGA CACTGTTGCG GCACCCCGGC GCCCTGGCGG AGTTGCGGGG CGACGGTACG
18421 CTGATGCCGC AGACGGTCGA CGAACTCCTG CGTTTCCACT CCATCGCGGA CGGCCTTCGA
18481 CGGGCGGTCA CGGAGGACAT CGAACTCGGC GGCATCACGC TGCGCGCCCC AGACGGCCTC
18541 ATCGTCTCGC TGGCCTCCGC CAACCGCGAC GAGAGCGCCT TCGCCTCCCC GGACGGCTTC
18601 GACCCGCACC ATCCGGCGAG CCGGCACGTC GCCTTCGGCT ACGGCCCCCA CCAGTGCCTG
18661 GGCCAGAACC TGGCCCGGCT GGAGCTGGAG GTCACCCTGG GCGCGGTGGT GGAGCGCATT
18721 CCCACGCTCA GGCTGGCCGG CGACGCCGAC GCACTGCGCG TCAAACAGGA TTCGACCATC
18781 TTCGGGCTGC ACGAGCTGCC GGTCGAGTGG TGACGGAAGG AGGACACAGC GTGCGGGTGA
18841 CAGTCGACCA GAGCCGGTGC CTGGGAGCCG GCCAGTGCGA GCAGCTGGCG CCGGAGGTCT
18901 TCCGCCAGGA CGAGGAAGGA CTGAGCCGGG TCCTCGTCCC CGAGCCCGAT CCGGCGTCAT
18961 GGCCGCGGGT GCTCCAGACG GTGGACCTCT GCCCCGTACA GGCCGTCCTC ATCGACGAGG
19021 GCCCCGGTCC CGCGCCGCAG GACACCAAGT GACCGCTGAC CGCTGGGCCG GCCGCACGGT
19081 GCTCGTCACG GGAGCACTCG GGTTCATCGG CTCCCACTTC GTCCGACAGC TGGAGGCGCG
19141 CGGAGCCGAG GTGCTCGCCC TCTACCGCAC CGAACGGCCG CAATTACAGG CCGAGTTGGC
19201 CGCGCTCGAC CGAGTACGCC TGATCAGGAC GGAGCTGCGG GACGAGTCGG ACGTGCGAGG
19261 GGCCTTCAAG TACCTGGCAC CCTCCATCGA CACCGTCGTC CACTGCGCGG CCATGGACGG
19321 CAACGCGCAG TTCAAGCTGG AGCGCTCGGC CGAGATCCTC GACAGCAACC AGCGGACCAT
19381 CTCCCACCTG CTGAACTGCG TACGGGACTT CGGCGTCGGC GAGGCCGTGG TCATGAGCTC
19441 CTCCGAGCTG TACTGCGCGC CGCCCACCGC GGCGGCCCAC GAGGACGACG ACTTCCGCCG
19501 ATCCATGCGG TACACGGACA ACGGCTACGT CCTGTCCAAG ACCTACGGCG AGATCCTGGC
19561 CAGGCTCCAC CGCGAGCAGT TCGGCACCAA CGTCTTCCTG GTGCGACCGG GCAACGTCTA
19621 CGGGCCGGGA GACGGCTACG ACCCCTCCCG GGGCCGGGTG ATCCCCAGCA TGCTGGCCAA
19681 GGCCGACGCC GGCGAGGAGA TAGAGATCTG GGGGGACGGC AGTCAGACCC GGTCCTTCAT
19741 CCACGTCACC GACCTGGTAC GGGCCTCACT GCGCCTGCTG GAGACCGGCA AGTACCCCGA
19801 GATGAACGTG GCCGGCGCGG AACAGGTCTC CATCCTGGAG CTCGCCCGGA TGGTGATGGC
19861 CGTCCTGGGA CGGCCCGAGC GCATCCGCCT CGACCCCGGC CGCCCCGTCG GCGCCCCGAG
19921 CAGACTTCTG GATCTGACCA GGATGTCGGA AGTGATCGAC TTCGAGCCCC AGCCCCTGCG
19981 GACCGGGCTG GAAGAGACCG CTCGCTGGTT CCGCCACCAC ACGCGCTGAA CCTCCTCTCA
20041 TACCCCCCTG GAAGGTAACT CGTGGTCACA CACGCCCCGA ACTCGCTGAT CAGTGACATA
20101 ATCCGCGCCT CCGGCGGGCA TGACGCCGAC CTCAAGGACC TGGCCGCCCG ACACGATCCG
20161 GCCGACATCG TCCGCGTACT CCTGGACGAG ATCACCTCAC GCTGCCCCGC TCCCGTGAAC
20221 GACGTCCCCG TCCTCGTCGA GCTGGCCGTC CGGGCGGGAG ACCGCCTCTT CCCCACCTAT
20281 CTGTACGTCC TCAAGGGCGG CCCGGTGCGC CTCGCGGCCA AGGACGAGGC GTTCGTCGCC
20341 ATGCGCGTCG AGTACGAGCT GGGCGAACTG GCCCGCGAGC TGTTCGGACC GGTGCGGGAG
20401 AACGTCACCG GCGTCCGCGG AACGACTCTC TTCCCCTACG TCGGGGACAC GGCGTCGGAA
20461 GGCGAGGAGG ACTCGGGTGC CGAGCACATC GGCACGCACT TCCTGGCCGC GCAGCAGGGC
20521 TCCCAGACCG TGCTCGCCGG CTGCCATTCC CACAAGCCGG ACCTCAGCGA GCTCTCCTCG
20581 CGCTACCTCA CCCCGAAGTG GGGCTCGCTG CACTGGTTCA CCCCCCACTA CGACCGCCAC
20641 TTCAGGAGCT ACCGGGACCA GCCCGTACGC GTTCTGGAGA TCGGCATCGG CGGCTACAAG
20701 CACCCCGAGT GGGGCGGCGG CTCCCTGCGC ATGTGGAAGC ACTTCTTCCA TCGCGGCGAG
20761 ATCTACGGCC TGGACATCGT CGACAAGTCG CACTTCGACG CGCCGCGCAT CACGACCCTG
20821 CGCGGCGACC AGAGCGACCC CGACCACCTG CGGTCGATCG CCGAGAAGTA CGGACCGTTC
20881 GACATCGTCA TCGACGACGG AAGCCACATC AACGACCACA TCCGGACCTC GTTCCAGGCA
20941 CTGTTCCCGC ATGTGCGGCC CGGCGGCCTC TACGTGATCG AGGACCTGTG GACCGCCTAC
21001 TGGTCCGGCT TCGGCGGCGA CGAGGACCCG AAGCGGTACA GCGGGACGAG CCTGGGCCTG
21061 CTCAAGTCCC TCGTCGACTC GATCCAGCAC GAGGAACTGC CGGAGGCCGG CGACCACCGT
21121 CCCAGTTACG CGGACCAGCA CGTGGTCGGC ATGCACCTCT ACCACAACCT GGCGTTCATC
21181 GAGAAGGGCA CCAACGCCGA GGGCGGCATC CCCCCGTGGA TCCCACGCGA CTTCGAGACC
21241 CTCGTCGCCG TCTCCTCCGG GGGCCACGCA TGAGGAGCCG TCGGCACCAG CCACCCGAAC
21301 ACACCCGACC GGACCGCAGG AGGCCCGCAT GCGCGTGACC CTGCTGAGCG TCGGATCCCG
21361 AGGCGACGTC CAGCCGTTCG TCGCCCTCGG CATCGGCCTC AAGGCCCGCG GCCACGACGT
21421 CACCCTGGCC GCCCCCGCCA CGCTGCGGCC ACTGGTCGAG CGCGCGGGAC TGACGTACAG
21481 GCTGTCCCCC GGGGATCCCG ACGGATTCTT CACCATGCCC GAGGTCGTCG AAGCGCTGCG

21541 GCGCGGCCCC TCGTTCAAGA ACATGCTCGC GGGGATGCCC GAGGCGCCCG AGAGCTACAC
21601 ACAGCAGGTC GTCGACGCGA TCCACGACGC CGCCGAGGGC GCCGACCTCA TAGTGAACGC
21661 GCCCCTCACC CTGGCCGCCG CGTACGGGCA CCCGCCCGCC CCGTGGGCCT CGGTGTCCTG
21721 GTGGCCCAAC AGCATGACCT CGGCCTTCCC GGCCGTCGAA TCCGGGCAGC GCCACCTCGG
21781 ACCGCTGACG TCCCTGTACA ACCGCTACAC CCATCGCAGG GCGGCACGCG ACGAGTGGGA
21841 GTGGCGACGC CCCGAGATCG ACGGCTACCG CCGACGCCTC GGCCTCCGGC CCTTCGGCGA
21901 CGAGTCCCCG TTCCTCCGAC TGGGGCACGA CCGCCCGTAC CTGTTCCCCT TCAGCCCCAG
21961 CGTGCTGCCC AAGCCGCGGG ACTGGCCGCG CCAGAGCCAC GTCACCGGCT ACTGGTTCTG
22021 GGACCAGCAC GGGGAGCCGC CCGCCGAGCT GGAGTCGTTC CTGGAGGACG GGGAGCCCCC
22081 GGTGGCGCTC ACCTTCGGCA GCACCTGGTC ACTCCACCGG CAGGAGGAGG CCCTCCAGGC
22141 CGCCCTCGAC GCCGTCCGTG GCGTCGGACG CCGACTGGTC ATGGTCGACG GACCGGACAG
22201 CGACCTGCCC GACGACGTGC TCCGCCTGCA CCAGGTGGAC TACGCCACCC TCTTCCCCAG
22261 GATGGCCGCG GTGATCCACC ACGGCGGCGC CGGCACGACC GCCGAGGTCC TCCGGGCCGG
22321 AGTGCCCCAG GTCATCGTGC CGGTCTTCGC CGATCACCCC TTCTGGGCGG CTCGACTGTC
22381 CAGAACAGGC GTCGCCGCCC GGCCGGTCCC CTTCGCCCGC TTCAGCCGAG AGGCACTGGC
22441 GCAGAGCGTC CGCCAGGCGG TCACCGATCC CGCGATGGCG GGCCGGGCCA GGCGACTGGG
22501 CGAACGGGTC TCCAAGGAAC GGGGAGTGGA CACCGCCTGC GACATCCTCG AGAAGTGGGC
22561 GGAGACGGCA CGCGCCACGG CCTGACACGG CCACCGGCGG GCGGGCCCGG AAGCCGCACG
22621 CCCGCCGGCC GACGGGTCCC GGGACCGCGC CGCTACGCCG ACAACCGGTA GGCGGAGAGC
22681 CGCACGGAGA GCGTGACCCG AGTCGGCGCC GGCAGCCGCT GGATCGTCTC CAGATCCCGC
22741 TGCGCGTCAC GGTGCCAGGC ATTGGGCCCC ATGCCGACCA GGTGCGCCAG AGCCTGGTGG
22801 TCGAGAGCCA TGGTCCGGGT CAACTCCTCC GTGGCGACGG CCGAGAAGTG CGGAGCGAGC
22861 TGCTCCGCGA GACGCGACTC CTTGCCTTCG TCCACCTGCA GCAGGCCGAG GGCGCCGATC
22921 ACCTCCCGCA GGTGATCGGG CAGAGGAGTG ACAACCAGGA GAACGCCACG GGGATGGAGA
22981 ACGCGATGCA GTTCAGGACC GTTGCGGGGA GCGAACGTGT TGATCACCAT GCCGGCTGCG
23041 GCATCCCGCA GCGGAAGCGT CTGCCAGGCG TCGGTCACCG CGGCCGCGAT CCGCGGATGC
23101 GCCTTCGCGG CACGCCGCAC GGCGTACTTG GAGATGTCCA GCAGCAGGCC CTGGGCATCG
23161 GGGAACGCTT CCATGACCCC GGCGTGATAG TGGCCCGTCC CCCCACCGAT GTCGACCACA
23221 CAGCCGGGCA CGGCCGGATC GGCCGTCCGC CGCGCCAGAT CGACCAGCGC ATCCATCACG
23281 GGGTCGTAGT GACCCGCCGA CAGGAATGCG TCCCGGGCCT CCACCATTTC CTTGGTGTCG
23341 GCCCGCAGCT TCGTCGCCCT GGGAAGCAGA TTCACATAAC CCTGCTTCGC GATGTCGAAG
23401 GAGTGTCCGG CGGGGCAGAA AAGTGCGCGG TCGCCCTGAG CCAGCGAAGC ACCGCAGTGC
23461 GGGCAGGCGA GGTAGCGCAC GATCCTGTTG AGCATGGGGC ACCGTTCCTT CGGGCGAGTC
23521 GGCAGACCGG CCAACCCTAA CGCAGGCGCC CGGCCGCCCA CCGCCCGGCG CAGGGCCGAC
23581 GAACCACCGT GACGTGCACC AGCCGCGCGT GAGAACTCCT CATGCGCGCA CCCTACGGAA
23641 ATCGGCAGGT CAACCGGCGA TTCCTGCGGG AATTCCGAGC GAAACGGCCT CACTGTGTTT
23701 CCCCGCTGCA TTTCCTCGCT GAATTCAGCG AATCCCGGCA GACGACCGGC TCTGCCGGCG
23761 TGACAGCCCC TATCGATCGA CCAGGAGTTT CGATGGCCCC GAAGAGTGGT GCGCAGCGTT
23821 CGAGCGACAT AGCCGTCGTC GGCATGTCCT GCCGCCTTCC GGGGGCACCG GGCATCGATG
23881 AGTTCTGGCA TCTGCTGACC ACCGGAGGCA GCGCGATCGA GCGTCGCGCC GACGGCACCT
23941 GGCGCGGCTC CCTGGACGGA GCCGCCGACT TCGACGCCGC CTTCTTCGAC ATGACCCCCC
24001 GCCAGGCCGC CGCCGCCGAC CCGCAGCAAC GACTCATGCT GGAACTCGGC TGGACGGCCC
24061 TGGAGAACGC CGGGATCGTC CCCGGCAGCC TCGCCGGCAC GGACACCGGC GTCTTCGTCG
24121 GCATCGCGGC CGACGACTAC GCGGCACTCC TGCACCGGTC CGCCACCCCC GTCAGCGGGC
24181 ACACCGCGAC GGGCCTGAGC CGGGGCATGG CCGCCAACCG CCTCTCCTAC CTCCTGGGCC
24241 TGCGCGGTCC CAGCCTCGCG GTGGACAGCG CGCAGTCCTC CTCGCTCGTC GCGGTCCACC
24301 TGGCCTGCGA GAGCCTGCGC CGCGGCGAGT CCGACCTCGC GATCGTCGGC GGCGTCAGCC
24361 TGATCCTCGC CGAGGACAGC ACGGCGGGCA TGGAGCTCAT GGGCGCGCTC TCGCCGGACG
24421 GCCGCTGCCA CACCTTCGAC GCACGCGCCA ACGGCTACGT ACGCGGTGAG GGCGGAGCCT
24481 GCGTCGTCCT CAAGCCCCTG GAGCGGGCAC TGGCCGACGG GGACCGCGTC CACTGCGTCG
24541 TCCGAGGAAG CGCGGTCAAC AACGACGGCG GCGGCTCCAC CCTGACCACC CCCCACCGCG
24601 AGGCCCAGGC CGCCGTCCTG CGGGCGGCGT ACGAACGGGC CGGGGTCGGC CCGGACCAGG
24661 TGTCCTACGT CGAACTGCAC GGTACGGGGA CGCCGGTCGG CGACCCCGTC GAGGCGGCGG
24721 CTCTCGGCGC GGTCCTCGGC ACGGCCCACG GCCGTAACGC CCCGCTGTCC GTGGGATCGG
24781 TCAAGACGAA CGTCGGCCAC CTGGAGGCGG CCGCGGGCCT CGTGGGATTC GTGAAGGCAG
24841 CCCTGTGCGT CCGCGAGGGC GTGGTGCCGC CGAGCCTGAA CCACGCGACG CCCAACCCTG
24901 CCATCCCCAT GGACCGGCTA AACCTGCGCG TACCCACGCG ACTGGAGCCC TGGCCGCACC

```
24961 CGGACGACCG AGCGACCGGC CGGCTGCGAC TGGCCGGCGT CTCGTCCTTC GGCATGGGTG
25021 GGACGAACGC GCATGTGGTG GTGGAGGAGG CGCCGCTTCC GGAGGCCGGG GAGCCGGTCG
25081 GGGCCGGTGT GCCTTTGGCT GTGGTGCCGG TGGTGGTGTC GGGTCGTTCT GCGGGTGCGG
25141 TGGCTGAACT GGCCTCCCGC TTGAACGAGT CGGTTCGTTC GGATCGGTTG GTGGATGTGG
25201 GGTTGTCGTC GGTGGTGTCG CGGTCGGTGT TCGAGCATCG GTCCGTGGTT CTGGCGGGGG
25261 ACTCTGCCGA GCTGAGTGCC GGTTTGGATG CTCTGGCCGC TGACGGAGTG TCTCCTGTCC
25321 TGGTGTCGGG TGTGGCGTCG GTCGGGGGTG GCCGGTCGGT CTTCGTGTTC CCGGGTGCGG
25381 GGGTGAAGTG GGCGGGGATG GCGCTCGGGT TGTGGGCGGA GTCTGCTGTG TTTGCGGAGT
25441 CGATGGCGCG GTGTGAGGCG GCGTTCGCCG GGTTGGTGGA GTGGCGTCTG GCGGATGTGC
25501 TGGGTGATGG GGCTGCGTTG GAGCGTGAGG ACGTGGTGCA GCCGGCGTCG TTCGCGGTGA
25561 TGGTGTCGTT GGCGGCGTTG TGGCGGTCGT TGGGTGTGGT GCCGGATGCG GTGGTGGGGC
25621 ATTCGCAGGG GGAGATCGCT GCTGCGGTGG TGGCGGGTGG TCTGTCGTTG GAGGACGGGG
25681 CGCGTGTGGT GGTGTTGCGT GCGCGGGTGG CTGAGGAGGT TTTGTCGGGT GGGGGGATTG
25741 CTTCGGTGCG TCTTTCGCGG GCCGAGGTGG AGGAGCGGTT GGCGGGTGGG GGTGGTGGGT
25801 TGAGTGTGGC GGTGGTGAAT GCGCCGTCGT CGACGGTGGT GGCGGGTGAG TTGGGGGATT
25861 TGGATCGGTT TGTTGCGGCG TGTGAGGCGG AGGGGGTGCG GGCGCGTCGG CTGGAGTTCG
25921 GGTATGCGTC GCATTCGAGG TTCGTGGAGC CGGTGCGTGA GCGGTTGTTG GAGGGGTTGG
25981 CCGATGTCCG TCCGGTGAGG GGGCGGATTC CGTTCTATTC GACGGTGGAG GCTGCGGAGT
26041 TCGATACGGC TGGTCTGGAT GCGGAGTACT GGTTCGGGAA TCTGCGTCGG CCGGTTCGCT
26101 TCCAGGAGAC GGTCGAGCGG CTGTTGGCGG ATGGTTTCCG GGTGTTCGTG GAGTGCGGCG
26161 CGCATCCGGT GCTGACCGGG GCGGTGCAGG AGACCGCGGA GACTGCGGGC CGGGAGATCT
26221 GCTCCGTCGG ATCCCTGCGT CGTGACGAGG GTGGACTGCG TCGCTTCCTG ACCTCTGCGG
26281 CGGAGGCGTT CGTCCAGGGG GTGGAGGTGT CCTGGCCGGT GCTGTTCGAC GGCACCGGCG
26341 CCCGGACGGT CGACCTGCCC ACCTACCCCT TCCAACGCCG GCACCACTGG GCACCCGACG
26401 GCTCCGCGTC GGCGGCGCCC ACACGGGACA TCCGACCGGA CGAGACCGCC GCGGTTCCAG
26461 CGGACACGAT GGACCTCGCG GGACAACTTC GCGCGGACGT GGCGTCGTTG CCCACCACCG
26521 AGCAGATCGC GCGGTTGCTC GACCAGGTAC GCGACGGTGT CGCCACGGTC CTCGGACTGG
26581 ACGCCCGGGA CGAGGTGCGC GCGGAGGCGA CGTTCAAGGA ACTGGGCGTC GAATCGCTGA
26641 CCGGCGTCGA GCTGAAGAAC CACCTCCGTG CCCGGACCGG ACTGCACGTC CCCACCTCGC
26701 TGATCTACGA CTGCCCGACT CCCCTCGCCG CCGCTCACTA CCTCCGCGAC GAGCTCTTGG
26761 GCCGACCCGC GGAGCAGGCC GTCGTCCCCG CCGGCATCCC GGTCGACGAA CCGATCGCGA
26821 TCGTGGGGAT GGGGTGCCGT CTGCCGGGTG GGGTGTCGTC GCCGGAGGGG TTGTGGGATC
26881 TGGTGGCGTC GGGGGTGGAT GCGGTTTCTC CGTTCCCCAC GGATCGGGGT TGGGATGTGG
26941 GGGGTCTGTT CGATCCGGAG CCGGGGGTGC CGGGGCGTTC GTATGTGCGT GAGGGCGGGT
27001 TCCTTCATGA GGCGGGGGAG TTCGACGCGG GGTTCTTCGG TATCTCTCCG CGTGAGGCGT
27061 TGGCGATGGA TCCGCAGCAG CGGTTGTTGC TGGAGACGTC GTGGGAGGCG TTGGAGCGGG
27121 CGGGCATCGA TCCGCACACG CTTCGCGGTT CACGGACCGG CGTCTACGCC GGAGTGATGG
27181 CCCAGGAATA CGGCCCCCGA CTCCACGAAG GCGCAGACGG ATACGAGGGC TATCTGCTGA
27241 CCGGATCCTC CAGCAGTGTC GCCTCGGGTC GTATCTCGTA CGTGCTGGGT CTGGAAGGGC
27301 CGGCGGTGAC GGTGGACACC GCGTGCTCGT CGTCGCTGGT CGCGCTGCAC CTGGCCGTGC
27361 GGGCGCTGCG CAGCGGTGAG TGCGACCTCG CCCTCGCCGG CGGCGCGACC GTCATGGCCG
27421 AACCCGGCAT GTTCGTGGAG TTCTCACGGC AGCGCGGGCT GTCTGCCCAC GGACGGTGCA
27481 AGGCGTACTC GGACTCGGCC GATGGCACGG GCTGGGCCGA GGGGGCGGGT GTGCTGCTCG
27541 TCGAGCGGTT GTCGGATGCG GTACGTCATG GGCGTCGGGT GCTGGCGGTC GTGCGTGGTT
27601 CCGCGGTCAA CCAGGACGGT GCGAGCAACG GACTGACGGC GCCGAACGGG CGGTCCCAGT
27661 CGCGTTTGAT CCGTCAGGCG TTGGCGGATG CGCGGTTGGG TGTGGCTGAT GTGGATGTGG
27721 TGGAGGGCCA CGGCACGGGG ACGCGTCTGG GTGATCCGAT CGAGGCGCAG GCGTTGTTGG
27781 CGACGTATGG GCAGCGGGAT GCGGGTCGGC CTCTGCGGCT TGGTTCGCTG AAGTCGAACG
27841 TGGGGCATAC GCAGGCGGCT GCCGGTGTGG CGGGCGTGAT CAAGATGGTC ATGGCGATGC
27901 GGCACGGTGT CCTGCCGAAG ACGCTGCACG TGGATGAGCC GACGGCGGAG GTGGACTGGT
27961 CGGCCGGCGC GGTGTCCCTG CTGAGGGAGC AGGAGGCGTG GCCGCGTGGC GAGCGTGTGC
28021 GGCGGGCCGG AGTCTCCTCG TTCGGCGTGA GTGGGACGAA CGCGCATGTG GTGGTCGAGG
28081 AGGCGCCGGT TCCGGAGGAC GGGGAGGCGA TCGAGGGCGG TGCGCCTTTG GCTGTGGTGC
28141 CGGTGGTGGT GTCGGGTCGT TCTGCGGGTG CGGTGGCGGA GCTGGCGGGC CGGGTCAGCG
28201 AGGTCGCTGG GTCTGGTCGG TTGGTGGATG TGGGGTTGTC GTCGGTGGTG TCGCGGTCGG
28261 TGTTCGAGCA CCGGTCCGTG GTGCTGGCGG GGGACTCTGC CGAGCTGAAT GCCGGTCTGG
28321 ATGCTCTGGC CGCTGACGGA GTGTCTCCTG TCCTGGTGTC GGGTGTGGCG TCGGGTGAGG
```

28381 GTGGCCGGTC GGTGTTCGTG TTCCCTGGTC AGGGGACGCA GTGGGCGGGG ATGGCGCTCG
28441 GGTTGTGGGC GGAGTCGGCG GTGTTCGCGG AGTCGATGGC GCGGTGTGAG GCGGCGTTCG
28501 CCGGGTTGGT GGAGTGGCGT CTGGCGGATG TGCTGGGTGA CGGGTCTGCG TTGGAGCGGG
28561 TCGATGTGGT GCAGCCGGCG TCGTTCGCGG TGATGGTGTC GCTGGCGGAG TTGTGGCGGT
28621 CGTTGGGTGT GGTGCCGGAT GCGGTGGTGG GGCATTCGCA GGGGGAGATC GCTGCTGCGG
28681 TGGTGGCGGG TGGTCTCTCG CTGGAGGATG GCGCGCGTGT GGTGGTGTTG CGTGCGCGGT
28741 TGATAGGCCG TGAGCTGGCC GGGCGCGGTG GGATGGCGTC GGTGGCGCTG CCCCTCGCGG
28801 TGGTGGAGGA GCGTCTGGCG GGGTGGGCGG GGCGTCTGGG TGTGGCGGTG GTCAACGGAC
28861 CCTCCGCCAC GGTCGTCGCG GGTGATGTGG ATGCGGTGGG GGAGTTTGTG ACCGCGTGCG
28921 AGGTGGAGGG GGTTCGGGCG CGTGTTCTGC CGGTGGACTA CGCCTCGCAC TCGGCGCACG
28981 TGGAGGACCT GAAAGCCGAG CTTGAACAGA TTCTGGCCGG CATCGGCCCG GTGACCGGTG
29041 GCATCCCGTT CTATTCGACG TCCGAAGCCG CGCAGATCGA CACGGCTGGT CTGGACGCGG
29101 GGTACTGGTT CGGGAATCTG CGTCGGCCGG TGCGGTTCCA GGAGACGGTC GAGCGGTTGC
29161 TGGCGGATGG TTTCCGGGTG TTCGTGGAGT GTGGCGCGCA TCCGGTGCTG ACGGGGGCGG
29221 TGCAGGAGAC CGCGGAATCC ACCGGTCGCC AGGTGTGTGC GGTCGGATCC CTGCGTCGTG
29281 ACGAGGGAGG TCTGCGCCGC TTCCTGACCT CTGCGGCGGA GGCGTTCGTC CAGGGGGTCG
29341 GGGTGTCCTG GCCGGCACTG TTCGACGGCA CCGGCGCCCG CACGGTCGAC CTGCCCACCT
29401 ATCCCTTCCA GCGTCGGCGT TACTGGCTGG AGTCACGTCC TCCTGCGGCG GTTGTTCCGT
29461 CGGGGGTCCA GGACGGATTG TCGTATGAGG TGGTGTGGAA GAGCCTGCCG GTACGGGAGT
29521 CGTCGCGTCT TGACGGCCGG TGGCTGCTCG TCGTGCCCGA AACCCTGGAC GCCGACGGCA
29581 CGCGGATCGC CCACGACCTC CAGCACGCCC TCACCACCCA CGGCGCCACG GTCTCCCGTG
29641 TGTCGGTCGA CGTGACGACG ATCGACCGCG CCGACCTGTC GGCGCGGCTC ACCACGAGCG
29701 CGGCCGAAGA CCAGGAACCG CTTGGGCGAG TGGTGTCCCT CCTGGGGTGG GCCGAGGGAG
29761 TACGGGCCCA TGGCCCGAAC GTACCGACTT CCGTCGCCGC CTCCCTGGCA CTCGTGCAGG
29821 CTGTCGGCGA TGCCGGGTTC GGTGTTCCGG TGTGGGCGGT GACGCGGGGT GCGGTGTCCG
29881 TGGTGCCTGG TGAGGTGCCG GAGACGGCGG GTGCGCAACT GTGGGCGCTC GGCCGGGTCG
29941 CCGGTCTCGA ACTTCCCGAC CGTTGGGGTG GTCTGATCGA TCTTCCGGCG GATGCCGATG
30001 CGCGTACGGC GGGGCTTGCG GTGCGGGCCC TGGCCGCCGG GATCGCCGAT GGTGAGGACC
30061 AGGTGGCGGT GCGCCCCTCG GGTGCCTACG GCCGGCGTGT AGTTCAGGCA GCCCACCGGG
30121 AGCCGTCGGG AGCGAAGACG GAGTGGCGAC CGCGTGGCAC CGTGCTCGTC ACCGGGGGAA
30181 TGGGCGCCAT CGGCACTCGG GTGGCCCGCT GGCTGGCCCG GAACGGAGCC GAACACCTGG
30241 TGCTGACCGG CCGCCGCGGT GCCGGGACCC CCGGCGCGGA CGAGCTGGCG GGAGAGCTGA
30301 GGGCGTCCGG AGTCCAGGTC ACGCTCGCCG CCTGCGACGT GTCCGATCGT GCCGCGCTGG
30361 CCGCGCTGCT CGACGCGCAT CCGCCGACCG CCGTCTTCCA CACGGCCGGT GTACTGAACG
30421 ACGGAACGGT CGACACGCTC ACTCCCGCTC ACCTGGACGG GGTCCTGAGC CCCAAGGCGA
30481 CGGCCGCCGT TCACCTGCAC GAGCTCACCG CCCACCTGGA CCTGGACGCC TTCGTCCTCT
30541 TCGCCTCCGT CACCGGCGTA TGGGGTAACG GCGGCCAGGC CGGGTACGCC ATGGCCAACG
30601 CGGCTCTGGA CGCGCTCGCC GAGCAGCGCC GTGCCGGCGG ACTTGCGGCG ACCTCCATCA
30661 GTTGGGGCCT CTGGGGTGGC GGCGGCATGG CCGAGGGTGA CGGCGAGGTG AGCCTCAACC
30721 GTCGTGGAAT CCGCGCTCTT GAGCCCGCCA CCGGCATCGA GGCGCTGCAG CGGACGCTCG
30781 ACCAGGGCGC CACCTGCCGC ACCGTCGTCG ACGTGGACTG GGGTCAGTTC GCTCCTCGTA
30841 CGGCGGCGCT GCGGCGCGGG CGGCTCTTCG CGGATCTGCC CGAGGTGCGG CGTGTCCTGG
30901 AGTCCGAGGG GGTTGCACGG GAGGACGCCG GAACCGTCGA GCCCGGCGCC GTGCTCGCCG
30961 AGCGCCTCGC ATCGCGCTCC GAGGCCGAAC AGCGACGCAT GCTCGTCGAG TTGGTACGAG
31021 CCGAAGCGGC TGCCGTCCTG CGTCACGACA CGACGGACCT CCTGGCGCCG CGCAGGTCGT
31081 TCAAGGACGC CGGGTTCGAC TCCTTGACCG CGCTGGAGCT CCGTAACCGG CTGAACACCG
31141 CCACCGGTGT CGTCCTTCCC GTCACCGTCG TCTTCGACCA CCCGAACCCC GGTGCACTGG
31201 CGGACTTCCT GTACGGCGAA GCACTGGGCC TGTCCGCGGC CAGGTCTTCC GCGAGCGATA
31261 CGGCCGACAC GACCCGCCCG GCCGCCGCCC CCGAAGAGCC GATCGCGATC GTCGGAATGG
31321 CCTGCCGCTA CCCGGGCGAG GCCCGTTCCC CCGAGGAACT GTGGAAGTTG CTCATCGACG
31381 AACGGGACGT CATCGGCCCC ATGCCCACGG ATCGGGGTTG GGATGTGGGG GGTCTGTTCG
31441 ATCCGGAGCC GGGGGTGCCG GGGCGTTCGT ATGTGCGTGA GGGCGGGTTC CTTCATGAGG
31501 CGGGGGAGTT CGACGCGGGG TTCTTCGGTA TTTCTCCGCG TGAGGCGTTG GCGATGGATC
31561 CGCAGCAGCG GTTGTTGCTG GAGACGTCGT GGGAGGCGTT GGAGCGGGCG GGCATCGATC
31621 CGCACACGCT CCGCGGCTCA CAGACCGGCG TCTACGCGGG GATGTTCCAC CAGGAGTACG
31681 CGACCCGGCT GCACGAGGCA CCCGTGGAGT TCGAAGGCCA CTTGCTGACG GGGACGTCCG
31741 GGAGTGTGGC TTCGGGTCGT ATCTCGTATG TGCTGGGTCT GGAGGGGCCG GCGGTGACGG

```
31801 TGGACACGGC GTGTTCGTCG TCGTTGGTGG CGCTGCATCT GGCGGTGCGG GCGTTGCGGA
31861 GTGGTGAGTG TGACCTTGCT CTTGCGGGTG GGGTGACGGT GATGGCGGAG CCGGGTGTGT
31921 TCGTGGAGTT CTCGCGGCAG CGGGGGTTGG CTGCGGACGG GCGGTGCAAG GCGTTCGCGG
31981 CTGCTGCCGA TGGCACGGGC TGGGCCGAGG GTGTGGGCGT GCTGGCGGTG GAGCGGTTGT
32041 CGGATGCGGT GCGTCATGGG CGTCGGGTGC TGGCGGTGGT GCGTGGCTCG GCGGTGAATC
32101 AGGACGGTGC GAGCAATGGG TTGACGGCTC CGAACGGTCC GTCGCAGCAG CGGGTGATTC
32161 GTCAGGCGTT GGCGGATGCG CGGTTGGGTG TGGCTCATCT GGATGTGGTG GAGGGGCATG
32221 GGACGGGGAC GCGTCTGGGT GATCCGATCG AGGCGCAGGC GTTGTTGGCG ACGTATGGGC
32281 AGCGGGATGC GGGTCGGCCT CTGCGGCTTG GTTCGCTGAA GTCGAATGTG GGGCATACGC
32341 AGGCGGCTGC CGGTGTGGCG GGCGTGATCA AGATGGTCAT GGCGATGCGG CACGGGGTCC
32401 TGCCGAAGAC GCTGCACGTC GATGAGGTCT CTCCGCACGT CGACTGGTCG GCGGGCGCGG
32461 TGTCCCTGCT GACGGAGCAG GAGCCGTGGC CCCGTGGTGA GCGCGTACGG CGGGCTGGTG
32521 TCTCCGCGTT CGGTGTGAGT GGGACGAACG CGCATGTGGT GGTGGAGGAG GCACCTGCTT
32581 CGGAGGCGCC GGTCGCGGTG GAGCCGGTGG AGCCGGGGGC TGTGGGGCTT CTTCCGGTGG
32641 TGCCCGTGGT GGTGTCGGGT CGTTCTGCGG GTGCGGTGGC TGAACTGGCC TCCCGCTTGA
32701 ACGAGTCGGT TCGTTCGGAT CGGTTGGTGG ATGTGGGGTT GTCGTCGGTG GTGTCGCGGT
32761 CGGTGTTCGA GCATCGGTCC GTGGTTCTGG CGGGGGACTC TGCCGAGCTG AGTGCCGGTT
32821 TGGATGCTCT GGCCGCTGAC GGAGTGTCTC CTGTCCTGGT GTCGGGTGTG GCGTCGGTCG
32881 GGGGTGGCCG GTCGGTGTTC GTGTTCCCGG GTGCGGGGGT GAAGTGGGCG GGGATGGCGC
32941 TCGGGTTGTG GGCGGAGTCT GCTGTGTTTG CGGAGTCGAT GGCGCGGTGT GAGGCGGCGT
33001 TCGCCGGGTT GGTGGAGTGG CGTCTGGCGG ATGTGCTGGG TGATGGGGCT GCGTTGGAGC
33061 GTGAGGACGT GGTGCAGCCG GCGTCGTTCG CGGTGATGGT GTCGTTGGCG GCGTTGTGGC
33121 GGTCGTTGGG TGTGGTGCCG GATGCGGTGG TGGGGCATTC GCAGGGGGAG ATCGCTGCTG
33181 CGGTGGTGGC GGGTGGTCTG TCGTTGGAGG ACGGGGCGCG TGTGGTGGTG TTGCGGGCGC
33241 GGGTGGCTGA GGAGGTTTTG TCGGGTGGGG GGATTGCTTC GGTGCGTCTT TCGCGGGCCG
33301 AGGTGGAGGA GCGGTTGGCG GGTGGGGGTG GTGGGTTGAG TGTGGCGGTG GTGAATGCGC
33361 CGTCGTCGAC GGTGGTGGCG GGTGAGTTGG GGGAGTTGGA TCGGTTTGTT GCGGCGTGTG
33421 AGGCGGAGGG GGTGCGGGCG CGTCGGCTGG AGTTCGGGTA TGCGTCGCAT TCGAGGTTCG
33481 TGGAGCCGGT GCGTGAGCGG TTGTTGGAGG GGTTGGCCGA TGTCCGTCCG GTGAGGGGGC
33541 GGATTCCGTT CTATTCGACG GTGGAGGCTG GGGAGTTCGA TACGGCTGGT CTGGATGCGG
33601 AGTACTGGTT CGGGAATCTG CGTCGGCCGG TTCGGTTCCA GGAGACGGTC GAGCGGTTGT
33661 TGGCGGATGG TTTCCGGGTG TTCGTGGAGT GTGGTGCGCA TCCGGTGCTG ACCGGGGCGG
33721 TGCAGGAGAC CGCGGAGACT GCGGGCCGGG AGGTGTGTGC GGTTGGTTCG CTGCGTCGTG
33781 ACGAGGGAGG TCTCCGTCGC TTCCTGACCT CTGCGGCGGA GGCGTTCGTC CAGGGGGTGG
33841 AGGTGTCCTG GCCGGTGCTG TTCGACGGCA CCGGCGCCCG CACGGTCGAC CTGCCCACCT
33901 ACCCCTTCCA ACGCCGCCAC TACTGGGCAC AGTCCTCGCC CGCCGGCGCC GGCAGCTCTG
33961 CTGCGGCCCG CTTCGGTATG ACCTGGGAGG AGCATCCGCT CCTCGGCGGC GCGCTGCCGC
34021 TCGCGGACTC CGGCGAGCTG CTGCTCGTCG GGAGGATCTC CCCGGCCTCC CACTCCTGGA
34081 TCGCCGACCA CACCGTGGCC GGGACCGCCC TGCTGCCCGG GACGGCCTTC GTCGACATGG
34141 CACTGCACGC CGCCGCGGTC GCGGGCTGTG CGGGTGTGGA GGAGCTGAGC ATCGAGGCCC
34201 CGCTGCCGGT GCACGGCGGG ATCCGGCTCC AGGTGGTGAT CGACGAGCCC GACTCCTCCG
34261 CGCGGCGCCG CGTGAGCGTG TTCGCGAGGC CGGAAGAGGA AGACGGGGAC GCCGGCCGCT
34321 GGACCCGACA CGCCACCGGC GTGCTGACCC CCGACGTCGC CCCCGAGCCG GGCCGGCCGC
34381 AGTGGTGCCG GCAGGCCTGG CCGCCGAGCG GCTCCGTCCG GGTGGAGGCG TCGGAGCTCT
34441 ACGACCGGTT CTCCGCGCTG GGATACGACT ACGGCGAGGT CTTCGCCGGG GTCGAGGCCG
34501 TCTGGCTGCG CGAGGGCGAG GCCTTCGCCG AGGTCCGCCT GCCCACGGGC GCGGCGCCCG
34561 ACGCCGAGCG GTTCGGGGTG CACCCCGGCC TCCTCGACGC GGCTCTGCAC CCCTGGCTGC
34621 TGGGCGACTT CCTGTCGCGG CCCGACGGCG GATCCGTACT GCTGCCGTTC GCGTGGCGCG
34681 GCATCACGCT CCACACGGCC GGTGCCGACG CGCTGCGGGT CCGTCTCGGA CCGGCCGGAG
34741 AAGGCGCTCT GTCGCTCGAA GCCGTCGACC TCTCCGGTGC CCCGGTGCTG TCGATGGACG
34801 CACTGGTGCT GCGTCCGCTC GCCCAGGACC GCCTGGCGGA ACTGGTCGGC GGCACGACCT
34861 CCACCCCGCT GTACCGCGTG GACTGGCAGC GGAGCCCGAT CGCGAGGACG GCGCCGTCGG
34921 CCACGGGGCT CTTCGGCTCC CTCCCGTCCG GTGCCGTCCG CCGCTGGGCG GTCGTCGGGC
34981 AGGGCGGTGT CGCCGCACGG TACGCGACGG CGGAACCCGG CACGGGGTGC GTCGGGGTCT
35041 TCCCCGACCT GGACGCACTG CGTACGACGC TGGACTCCGG AGCGGACGGC CCCGACATCG
35101 TCCTCGCCGA CTTCGGGGCC CGGCCAGGCG ACGCCGCGCC GCACGGGACG GATCCGGCCG
35161 ACGGCGCACG CGACACGGTC CGGCGGGGGC TCGCCCTCAT ACAGGGCTGG CTGTCCGACG
```

35221 AGCGCTTCGC CGCCGCGCGT CTCGCCGTGC TCACCGAGCA CGCCGTCGCC ACCGAGGCGG
35281 ACACCCGCCG GACGGACCTC GCGGGCTCGG CACTGTGGGG GCTGATGCGT TCGGCGCAGA
35341 CCGAGCACCC CGACCGCTTC GTCCTCGTCG ACCACGACGG GCAGGACGCC TCGTACCGGA
35401 CGCTGCCCAC CGCGCTCGAC AGCGAAATCC CGCAACTGGC GCTCCGAGCC GGGGAGACGC
35461 TGGCCCCCGA ACTGGCGGTC CTGCCGAGTC CGGCCGACGG GGGGCCCGCG ACAAGCGCGG
35521 CGTTCGATCC CGAAGGCACG GTACTCGTCA CCGGAGCCAC CGGCACCCTC GGCAGCCTGC
35581 TGGCCCGGCA CCTGGTCACG GCACACGGCG TACGGCATCT GCTGCTGCTC ACCCGCAGCG
35641 GACGCGAAGC CGCGGGGGCC GCCGAACTGG AGCGTGAACT CCGTCAACGG GGAGCCGAGT
35701 TCCAGCTCCT CTCCTGTGAC GCGACGGACC GGGCAGCGCT GAAGGAGGCC CTCGCCACCG
35761 TCCCCGCCGC CCACCCGCTG ACCGCGGTGA TCCACACGGC CGGCGTCCTC GACGACGGCG
35821 TCGTCGAGGC GCTGACCCCC GAACGGCTGG ACCGCGTGCT GCGCCCCAAG GCGGACGCCG
35881 CGCTGAACCT GCACGACCTG ACCGAGGGAA TGCCGCTGAA GGCGTTCGTC CTGTACTCCG
35941 GGGCGGTCGG ACTGCTGGGT GGAGCGGGCC AGGCCAACTA CGCGGCAGCC AACGCGTTCC
36001 TGGACGGCCT GGCCCAACAC CGGCACGCGC AAGGGCTGCC CGCGGTGTCC CTGGCATGGG
36061 GACTCTGGAG CGCCACCAGC ACGTTCACCG ACCATCTCGG CGAGGTGGAC CTGCGGCGCA
36121 TGGAGCGGTC CGGCATCACG CCGCTCACGG ACGAGCAGGG CCTTGACCTG TTCGACCGGG
36181 CCCTCGGCGC CGCGGTGGAC GCGCCGCAGC TCTGCGTGAT GGGGCTGGAC ACGGCGGCGC
36241 TGCGCCGGCA GGCGGCCGAG CACGGGCCGA CTTCGATGCC TCCTCTGCTG CGTACGCTGG
36301 CGGCGCCTCC CGTACGGCGC GGCGCGGGGC GCTCCGGCCG GGGCGGACGG GCGGCGTCCG
36361 CCACGGACGC GCCGAGCCGG GCGCAGGCCC TGCGCGAGCG ACTGACGGGC CTGGACGCGG
36421 CGGCACGGCG GGACGAACTC CTGGTCCTGT CGCAGGCGCA GTTGGCCGAT GTGCTGGGCT
36481 TCGCCGACAA GACCGCGGTG GACCCCGTTC GTTCCTTCCG CGAGATCGGT CTGGACTCGC
36541 TGACCGCCGT CGAGCTGCGC AACCGGCTCG GTGTCGTCAC CGGACTGCGG CTGCCGCCGG
36601 CGCTGGTCTT CGACCACCCC AACCTCGACG CGCTCGCGGC CCACCTGGCG GAGCTCCTCG
36661 CGGCTGAGGG CCGGGACGAC GCGGGCGCCG CGGCGCTCTC GGGAATCGAC GCCCTGGACC
36721 GGGCGGTCCG GGAGATGGCG GCCGACGACA CGCGCCGTGA CGCCGTCCGC CGACGCCTCG
36781 CGGAACTGCT GGCGGTGGTC GGCGACGCCC CGCGGGACGG CGGCCGCGCC CCACGGGCGG
36841 CCGCCGACGC GGGAGGCCGC GACGCTCAAG CGGACCCCGA CCTGCTGGGC CGGCTGGACT
36901 CCGCCTCCGA CGACGATCTG TTCGCGTTCA TCGAAGACCA GCTGTGAGCG GGACGCCGCG
36961 CGCGTTCCCC ACCCGTCCCT AAGCGCCGCA TCAGGCGCAC TCGCACCGAC ACGAGCACGC
37021 AGGCCAGGAG GGTCCGGTCG ATGACGGCCA ACGATGACAA GATCCGCGAC TACCTGAAGC
37081 GGGTCGTCGC CGAGCTGCAC AGCACGCGGC AACGGCTCAA CGCCTTGGAG CACGACGCCC
37141 GCGAGCCCAT CGCCATCGTG GGGATGAGCT GCCGGCTGCC CGGCGGGGTG ACCACCCCCG
37201 AGAGCCTGTG GAGGCTGGTC GACTCCGGCA CCGACGCCGC CTCGCCGTTC CCCGACGACC
37261 GGGGCTGGGA CCTGGACGCG CTCCACCATC CGGAGTCGGG AGCCGTCCAC TCCCGCGAGG
37321 GCGGATTCCT CCACGACAGC GCGGACTTCG ACGCGGAGTT CTTCGGCATC AGCCCGCGAG
37381 AGGCCCTGGC CATGGACCCG CAGCAGCGGC TGCTGCTGGA GACCGCCTGG GAGGTGTTCG
37441 AGCGCGCCGG CATCGACCCG GTCTCCGCCC GCGGCTCCCG CACGGGGGTG TACGCGGGCG
37501 TCATGTACCA CGACTACGGC GCCCGGCTGA ACGAGATCCC GCCGGGCCTC GAGGGCTACC
37561 TGGTCAACGG CAGCGCGGGC AGCATCGCCT CGGGCCGGGT GGCCTACACC CTCGGTCTGG
37621 AGGGCCCCGC CGTCACCGTC GACACGGCCT GCTCCTCGTC ACTGGTCGCC GTGCACCTGG
37681 CGGCACAGGC ACTGCGGCGG CGGGAGTGCG ACATGGCGCT CGCGGGCGGC GCGACCGTCC
37741 TGTCCACCCC CGACCTGTTC ATCGACTTCG CGCGACTCGG CGGCCTGGCC TCCGACGGGC
37801 GCTGCAAGGC GTTCTCCGAC GCCGCCGACG GCACCAGCTT CGCCGAGGGC GCCGGCCTGC
37861 TGCTCCTCAT GCGGCTGTCG GACGCGGTGG CCGAGGGCCA CACCGTCCTG GCGGTCGTCC
37921 GAGGCTCCGC CGTCAACCAG GACGGGGCGA GCAACGGCCT GACGGCCCCC AACGGCCTCG
37981 CCCAGCAACG CGTGATCCGC GAGGCGCTCG CCGACGCGGA CCTGGACCCC GACCAGATCG
38041 ACGCGGTGGA GGCGCACGGC ACCGGAACCA GGCTCGGCGA CCCCATCGAG GCGCAGGCCC
38101 TGCTGCACAC GTACGGCACG AGCCGCAGCC CCGAACGACC CCTGTGGCTC GGTTCGCTGA
38161 AGTCCAACAT CGGCCACACC CAGGCCGCCG CCGGAGTGGC CGGAGTCATC AAGACGGTGC
38221 TGGCGATGCG CCACGGACGG CTGCCCCGCA CACTGCACGT CACCCGCCCC TCCAGCCGGG
38281 TGGAATGGTC GGCGGGCGCG GTGGAACTGC TCACGCGGGC ACAGGACTGG CCCGGCCAGG
38341 GGAACGCCCC GCGCCGCGCC GGAGTGTCGT CCTTCGGTGC CAGCGGCACC AACGCACACC
38401 TGATCCTGGA AGGCGTCCCG GACGGCGACA TCACGGTCGC GGAGACCCGA CCGGCCACCG
38461 GCGGCGGCGC CTGGCCGCTC GCGGGCCGGA CCGAAGCGGC CCTGCGCGCA CAGGCCCGGC
38521 GGCTCCACGA CCACCTCGCC GCCCGCCCCC ACGTCTCACC CGCCGCGGTC GGGCGGACGC
38581 TGGTCCGCTC CCGCACGGCG TTCGAGCACC GGGCCGTCGT CCTCGGACAG GACACCGCCG

38641 ACCTCCTGAG CGGCCTCGCG GAGCTGGCGT CCGGCGGCGC TCACGGACCC GGCGTGATCA
38701 CCGGCCGGGC CGCGCGCGGC CGCCGTACCG CACTGCTCTT CACCGGACAG GGCAGCCAGC
38761 GGCCGGGTGC CGGACGGCAT CTCTACGAGC GGTACGAGGT GTTCGCCCGC GCCCTGGACG
38821 AGACGGCCGC GGCACTCGAC CGGCACCTCG ACCGCCCGCT GCGCGACGTG ATGTTCGCGG
38881 AGCCGGGCGG CGCGACGGCC GGACTCCTCG ACCGCACCGA GTACACCCAG CCCGCACTGT
38941 TCGCCCTGGA AGTCGCCCTC TTCCGACTGG TGACCGCCGG GGGCCTGCGC CCCGACGCAC
39001 TCCTCGGGCA CTCCGTCGGC GAACTGGCCG CCGCCCACGT CGCCGGAGTG TTCACCCTGC
39061 CCGACGCCGC CCGCCTGGTG ACGGCGCGAG GCCGACTGAT GGGCGAGCTG CCGGCCGGTG
39121 GCGCCATGAT GGCGATCCAG GCGAGCGGCC CGGAGATCGA GGAGACGATC ACGGCGCTCG
39181 CGGCCCACCG GTCGGCGCGC GTCGCCGTCG CCGCACTCAA CGGTCCCGAC GCCACCGTGA
39241 TCTCGGGCGA CGAGGACGTG GTCGCCGAAC TCGCCACGCT GTGGCGGGAG CGGGGCCGCC
39301 GCACCAGGGC GCTGCCCGTC AGCCACGCCT TCCACTCGCC GCACATGGAC GCCGCACTGG
39361 AACCGTTCGC CCGGATCGCG CGCGACGTCT CCTACGCCGA ACCGCGCATC CCGGTGGTCT
39421 CCAACCTGAC CGGCGGCATC GCGTCGGCCA CGACGCTGTG CGCCCCCGAG TACTGGGTGC
39481 GCCACGCGCG CGAGGCCGTG CGCTTCAGCG ACGGCTTCCG CGCCCTGCGC GACCAGGGGA
39541 TCGACACCTT CATCGAGCTC GGACCGGACG GCGTGCTGTC CGCCCTGGGC CGCGACTGCC
39601 TCCGCGAGGA GGAAGGAGAC GCCCCACGCC AGGACGGCTC GGCGGACCCC GACACGACCG
39661 GCTCCCGCGC CGACGGGGGG CGGCGACCCG TGCTGACGGT GCCGCTGCTG CGCCGGGACC
39721 GCGACGAGAC GACCACCTGC CTCGGGGCCC TGGCCACCGT CCACACCCAC GGCGTCCCCG
39781 TCGACCTCGC GGCCGTGCAC GGCGCCCCCG AGGGGCCCGC CGTCGAGCTC CCCACCTACG
39841 CCTTCCAACG CACGCGCTAC TGGCTGGACG CCCCGGCCCC CGCCGCCGGC CCCACCGCCA
39901 CCGGTCTGGA GGCGACGGAC CAGCCCCTGC TCCCGGCCGT CATCGACCTG CCCGACGGGG
39961 AAGGCACCGT ACGGACCGGA CTGCTCTCCC TGCGCACCCA TCCGTGGATC GCCGACCACC
40021 GCGTCCGCGA CCACGCCGTG GTGCCCGGAG CGGCCCTGCT GGACGTCGCC GCCTGGGCGG
40081 GCACCGAAGC GGGCTGCCCC CGGGTCGCCG AACTGACCTT CGCCACGCCC CTCGTCCTGC
40141 CCGAGAACGG AGAAGGAGTC CGACTGCGCG TGACGGTCAG CGGCCCCGAC GCGGAAGGCA
40201 TCCGTTCGCT ACGCATCGAC TCCCGGCCCG CCGACACGGT CCGTACCGCC GATGCCCCCT
40261 CCGACTGGAC CCGCCACGCG TCCGGCACCC TCGTCCCCGC ACCCGAGGAG GCCGGCGACG
40321 GCACCGGCGT GCCGACCGAA CTGCTCGGCG CCTGGCCCCC GGCCGACGCG ACCCCGGTCG
40381 CCCTCGACGC GGACGCCGTC GCGGCCGAGT ATCAGCGCCT CGCGGCCGGC GGCGTGACGT
40441 ACGGCCCCGC GTTCCGGGCA CTGCGCGCCG TCTGGCGCCG CGGCGCAGAG GTCTTCGCCG
40501 AGGTCCGGCT TCCCGGCCAG GCGGCCGCCG ACGCCTCGCG GTACGGCATG CACCCGGCCC
40561 TGCTGGACGC CCTGACGCAC GCCACCGGGT TCGGCGAGCG GTCCACCGAG GCCCGCGGCC
40621 TGGTCCCGTT CGCCTGGAGT GACGTCCGGA TCCACGTCCG CGGCGCCGAC TCCCTGCGCG
40681 TACGCATCGC GCCGGCCGGC CCCGACGCCG TGACCGTCGC CGCGGTCGAC CCGACGGGCC
40741 GCCCGGTCCT CGCCGCCCGC TCGCTCACGC TGCGCCCCCT GGCGGAGAGC CGGTTCCAGG
40801 ACCCGGAGGC GGACAGCACG CCGCTGTACC GACTGGAGTG GACACCGGCC CCCGGTTCCG
40861 TGACCGGGCA CGCCGGTCCA AGGCAGGCGG CAGCGGAGTG GGGCGTCCTC GGCGACCCGG
40921 TCCAGGCCCT CCTCGACGCC GTGCGCGACG GGGCGGAGGC ACCCGTGCGA ACCCATGACG
40981 ACCTGCTCGC GCTCGCGGCC TCCGACACGG CCCCGCCCGA CCATGTGCTG GCGCTGCTGG
41041 GCCACGACGG GGACGCTCTC GCCACGGGCG CCCACGACCT GGCCGCACGC GCCCTGGCCC
41101 TGGTACAGGG CTGGCTGACC CACGCCCGCT TCGCCGACGC GCGACTGGTC GTGCTGACGC
41161 AGGGGGCGGT GACGGCCGGC ACGAGCCCCG TCCACCCGGC CGCGGCCGCC GCCTGGGGGC
41221 TGCTGCGCAG CGCACAGTCC GAGCACCCGG GCCGCTTCGT CCTCGTCGAC GCGGACCCCG
41281 CCGACCCGGC CGCCTCGTAC CGTTCCCTGC CACGGGCCGT CGCCTCCGGG GCGTCCCAAC
41341 TCGCCCTGCG CGGCGCCGAG ATCCTCGTCC CCCGGCTCGC CCGGGGAACG GACCGACAGG
41401 CCACCGTGCC CGGACACCCC GGCGACGTCA CCGCACCGGA GACGACCGCT GCCCCCGAGC
41461 CGGCCCCGTC CGGCACCCCC TCCGGCCCCT GGCCCGCGGA CGGCACCGTG CTCGTCACGG
41521 GCGGAACCGG GACCCTGGGC AAGGCCGTGG CCCGGCACCT CGTGACCAAG CACGGTGTCC
41581 GGCACCTGAT CCTGGCCGGT CGGCGAGGCG CGGACACCCC CGGGGCGGCC GACCTGGCCA
41641 CCGAACTGAC CGGCCTGGGC GCCACCGTGA ACATCGTCCG CTGCGATGCC GCCGACCGCT
41701 CGGCGCTCGA AGGCGTCCTG GCCGCCGTCC CCGCCGCGCA CCCGCTCACC GCCGTCGTGC
41761 ACACCGCCGG AGTGCTCGAC GACGGCATCG TCACGGCGCA GACGCCCCGA CGCCTCTCGG
41821 CGGTCCTGCG CGCCAAGGCG GACGCGGTCA GCCACCTGCA CGAACTGACC CGCGACCTGG
41881 ACCTGTCCGC CTTCGTCCTC TTCTCATCGG CCGCCGGAAC CCTCGGCAGC CCCGGCCAGT
41941 CCGGCTACGC GGCCGCCAAC AGCTTCCTCG ACGCGTTCGC CGCCTGGCGG CGAGCGCAGG
42001 GCCTCCCCGC CGTGTCCCTG GCATGGGGAC TGTGGGGTGA CGGCGGTGAC GGTCGTGACG

42061 GCGGTGGCTC GGCGGCCGAC GGCATGGGAG CGAGCCTGGC CGCCGCCGAC CTGGCACGGC
42121 TGCGCCGTTC CGGCATCCTC CCGCTCGACC CGGCCGAAGC GCTGCGCCTG TTCGACGAGG
42181 CCTGCGACCC CGCCAGGACC GAGGCCGTAC TGCTGCCGAT CCGCCTCGAC CTGACCGGCC
42241 TGCGCGCCCG TTCCGCCCGC GGCGCCGTAC ACGCGAGCGT GGTGCCCGAA GTGCTGCACA
42301 CCTTGGTGCC CCCGCCCGCC GGTGCCGGAT CCCCGGCCGG TGCCGACGCG TCGGATCCCG
42361 CGGCGGGCCA GGCGCCCCCG GCCCCGGCGT CCGACACCCT GGCCGAACGG CTCGCCGGGA
42421 AGCCCCGAGG CGAACGGCTC ACCGCCCTCA CCCAACTGGT ACGCACCGAG ATCGCCTCGG
42481 TACTCGGGCA CCCCGACTCC GGCCGCGTCC AGCTCCAGTC CTCCTTCAAG GAGTCCGGCT
42541 TCGACTCGCT CACCGCCGTC GAACTCCGCA ACCGGCTCAC CGCGGCCACC GGAACGAAGC
42601 TTCCCGCCAC CCTCGTCTTC GACCATCCGA CACCCGCGGC ACTCGTCGAC CACCTGGAAC
42661 AGGAACTGCC GAAGGCAGCA CAGGAGATCC CGGCGGACCT CCCGGCCGTT CTCGACGCAC
42721 TCGACCGGAT CCGGGACGGA CTCGCCACCG CCGCCACCGA CGACAGCAGC CGCGACCACA
42781 TCGCGGAGCG TCTCCAGGCG TTGCTCGGCA CGCTCACCTC GGCTGCGGGC GTCAGCCGCC
42841 CGACCGGCAG CCCGGGCGAG CACGACCGGC AGGGCCCCGA TGAGCTGTCG CTCGGCCAAC
42901 GACTCGCCGC CAGCAGCGAC GACGAACTCT TCGACCTCTT CGACAGCGAC TTCCGATCGA
42961 CGTAGCCCAG GGCCACCCTT CCGCCTCCGC CGCCCGCCCC ACACCCCTGG AGAACAGCAC
43021 GATGTCTTCC ACATCGCCCG CCACCAACGA AGAGAAGCTC CGCGACTACC TCCGCCGCGC
43081 CATGACCGAC CTGCACGAGG CCCGCGAGCA GATCCGCCGG ACCGAGTCGG CCAGGCACGA
43141 GCCGATCGCG ATCGTGGGGA TGGGGTGCCG TCTGCCGGGT GGGGTGTCGT CGCCGGAGGG
43201 GTTGTGGGAT CTGGTGGCGT CGGGGGTGGA TGCGGTTTCT CCGTTCCCCA CGGATCGGGG
43261 TTGGGATGTG GGGGGTCTGT TCGATCCGGA GCCGGGGGTG CCGGGGCGTT CGTATGTGCG
43321 TGAGGGCGGG TTCCTTCATG AGGCGGGGGA GTTCGACGCG GGGTTCTTCG GTATCTCTCC
43381 GCGTGAGGCG TTGGCGATGG ATCCGCAGCA GCGGTTGCTG TTGGAGACGT CGTGGGAGGC
43441 GTTGGAGCGG GCGGGCATCG ATCCGCACAC GCTCCGCGGC TCACGGACCG GCGTCTACGC
43501 CGGAGTGATG TACCACGACT ACGGCAGCAC CGCGACCGTC TCCGTCGCCT CCGACGACGA
43561 GACCGCCGGA TTCCTCGGCA CGGGGACGTC CGGGAGTGTG GCTTCGGGTC GTATCTCGTA
43621 TGTGCTGGGG CTGGAGGGGC CGGCGGTGAC GGTGGACACG GCGTGTTCGT CGTCGTTGGT
43681 GGCGCTGCAT CTGGCGGTGC GGGCGCTGCG GAGTGGTGAG TGTGACCTTG CTCTTGCGGG
43741 TGGGGTGACG GTGATGGCGG AGCCGGGTGT GTTCGTGGAG TTCTCGCGGC AGCGGGGGTT
43801 GGCTGCGGAC GGGCGGTGCA AGGCGTTCGC GGCTGCTGCC GATGGCACGG GCTGGGCCGA
43861 GGGTGTGGGC GTGCTGGCGG TGGAGCGGTT GTCGGATGCG GTGCGTCACG GGCGCCGGGT
43921 CCTGGCGGTC GTGCGTGGTT CCGCGGTCAA CCAGGACGGT GCGAGCAATG GGTTGACGGC
43981 TCCGAACGGT CCGTCGCAGC AGCGGGTGAT TCGTCAGGCG TTGGCGGATG CGCGGTTGGG
44041 TGTGGCTGAT GTGGATGTGG TGGAGGGGCA TGGGACGGGG ACGCGTCTGG GTGATCCGAT
44101 CGAGGCGCAG GCGTTGTTGG CGACGTATGG GCAGCGGGAT GCGGGTCGGG CTTTGCGGCT
44161 TGGTTCGCTG AAGTCGAACG TGGGGCATAC GCAGGCGGCT GCCGGTGTGG CGGGCGTGAT
44221 CAAGATGGTC ATGGCGATGC GGCACGGTGT CCTGCCGAAG ACGCTGCACG TGGATGAGCC
44281 GACGGCGGAG GTGGACTGGT CGGCGGGCGC GGTGTCCCTG CTGAGGGAGC AGGAGGCGTG
44341 GCCTGAGGTG GGGCGTCTGC GTAGGGCTGC GGTGTCTTCG TTCGGTGTGA GTGGGACGAA
44401 CGCGCATGTG GTGGTGGAGG AGGCACCTGC TTCGGAGGCG CCGGTCGCGG GGGAGCCGGT
44461 GGAGCCGGTG GAGCCGGGGG CTGTGGGGCT TCTTCCGGTG GTGCCGGTGG TGGTGTCGGG
44521 TCGTTCTGCG GGTGCGGTGG CGGAGCTGGC CTCCCGCTTG AACGAGTCGG TTCGTTCGGA
44581 TCGGTTGGTG GATGTGGGGT TGTCGTCGGT GGTGTCGCGG TCGGTGTTCG AGCACCGGTC
44641 CGTGGTTCTG GCGGAGGACT CTGCCGAGCT GCATACCGGT CTGGTTGCTG TCGGGACTGG
44701 GGTGCCGTCG CCTGGCGTGG TGTCGGGTGT GGCGTCGGTC GAGGGTGGCC GGTCGGTGTT
44761 CGTGTTCCCT GGTCAGGGGA CGCAGTGGGC GGGGATGGCG CTCGGGTTGT GGGCGGAGTC
44821 GGCGGTGTTC GCGGAGTCGA TGGCGCGGTG TGAGGCGGCG TTCGCCGGGT TGGTGGACTG
44881 GCGTCTGGCG GATGTGCTGG GTGACAGGTC TGCGTTGGAG CGGGTCGATG TGGTGCAGCC
44941 GGCGTCGTTC GCGGTGATGG TGTCGCTGGC CGAGCTGTGG CGGTCGCTGG GTGTGGTGCC
45001 CGATGCGGTG GTGGGGCATT CGCAGGGGGA GATCGCTGCT GCGGTGGTGG CGGGTGGTCT
45061 CTCGCTGGAG GACGGCGCGC GTGTGGTGGT GTTGCGTGCG CGGTTGATAG GCCGTGAGCT
45121 GGCCGGGCAC GGTGGGATGG CGTCGGTGGC GCTGCCGGTC GCGGTGGTGG AGGAGCGTCT
45181 GGCGGCGTGG GCGGGGCGTC TGGGTGTGGC GGTGGTCAAC GCACCCTCCG CCACGGTCGT
45241 CGCGGGTGAT GTGGACGCGG TGGCGGAGTT TGTGACCGCG TGCGAGGTGG AGGGGGTTCG
45301 GGCGCGTGTT CTGCCGGTGG ACTACGCCTC GCACTCGGCG CACGTGGAGG AGCTGAGGGC
45361 CGAGCTTGAA CAGATTCTGG CCGGCATCGA CCCGGTGGCC GGTGAGACCC CCCTGTACTC
45421 CACGGTGGAG GCGGGTGTCG TGGATACGGC GTCGATGGAT GCGGGGTACT GGTTCAGGAA

```
45481 TCTGCGTCGG CCGGTTCGTT TCCAGGAGAC GGTCGAGCGG TTGCTGGCGG ATGGTTTCCG
45541 GGTGTTCGTG GAGTGCGGCG CGCATCCGGT GCTGACGGGG GCGGTGCAGG AGACCGCGGA
45601 ATCCACCGGT CGCCAGGTGT GTGCGGTCGG ATCCCTGCGT CGTGACGAGG GTGGTCTGCG
45661 ACGCTTCCTG ACCTCTGCGG CGGAGGCGTT CGTCCAGGGG GTGGAGGTGT CCTGGCCGGT
45721 GCTGTTCGAT GGCACCGGCG CCCGCACGGT CGACCTGCCC ACCTACCCCT TCCAGCGTCG
45781 GCGTTACTGG CTGGAGTCAC GTCCTCCTGC GGCGGTTGTT CCGTCGGGGG TCCAGGACGG
45841 ATTGTCGTAT GAGGTGGTGT GGAAGAGCCT GCCGGTACGG GAGTCGTCCC GTCTTGACGG
45901 CCGGTGGCTG CTCGTCGTGC CCGAAACCCT GGACGCCGAC GGCACGCGGA TCGCCCACGA
45961 CCTCCAGCAC GCCCTCACCA CCCACGGCGC CACCGTGCAC ACTCTTGCTC TTGACCCCAG
46021 CGCGGCGCAC TTCGACGGTC TCTTTGACGG GATACTCCAG GAAGAAACAG ATGTCACGGG
46081 CATCTTCTCT CTCCTCGGAC TGGCATCGGG CCCGCACCCG GATCACGGCG AGGTGGAGCT
46141 CGCGGGAGCC GCGTCGCTGA CGTTGATGCG CCAAGCCCAG CGAGACGGCT TCCGTGCTCC
46201 GGTGTGGGCG GTGACGCGGG GTGCGGTGTC CGTGGTGCCT GGTGAGGTGC CGGAGACCGC
46261 GGGTGCGCAA CTGTGGGCGC TCGGCCGGGT CGCCGGTCTC GAACTCCCCG ACCGTTGGGG
46321 TGGTCTGATC GATCTCCCGG CGGATGCCGA TGCGCGTACG GCGGGGCTTG CGGTGCGGGC
46381 CCTGGCCGCC GGGATCGCCG ATGGTGAGGA CCAGCTGGCG GTGCGCCCCT CAGGTGCCTA
46441 CGGCCGGCGC CTCGTACGAG CCACCGCGCG CCGGGGACGG AAGGACTGGC GCCCGCAGGG
46501 TACGGTGCTG CTCGCCGGGC ACCTCGACGC CGTCGGTGAA CCACTGGCCC GATGGCTGCT
46561 CACCGGCGGC GCGGACCACG TCGTCCTTGC GGATCCCGCC CTGACCGAAC TCCCGGCCAC
46621 CCTCGCGGAT CTGGCCCAGA CCGTGACGAC CGCTGCGGCA CCCGACCTTG CCGACCGTGC
46681 AGTCCTCGCC GCCCTGGTCA CCGAGTACGT ACCCGCCACC GTGGTCGTCG TTCCGCCCGC
46741 GGCGGAGCTC GCTCCGCTGG CGAGTATCAG CCCGGCCGAC CTCGCGGCGG CCGTCACCGC
46801 CAAGTCCGCG ACCGCGGCGC ACTTCGACGC GCTGCTCGAC GGACCCCACG CACCGGAGCT
46861 GGTGCTGATC TCCTCGGTCG CGGGGATCTG GGGTGGTGTC CGGCAGGGTG CGTACGCCGT
46921 CGGTGCCGCT CACCTCGATG CCCTGGCCGC CCGCCGCAGG GCCCGCGGTC TGTCGGCGGC
46981 CTCCGTCGCG TGGACGCCCT GGGCGGGTTC CGTCACCGCG GACGGCTCCG CCGCCGAGTC
47041 GCTGCGGCAG TACGGCATCG CTCCGCTGGA GCCGCAGGCG GCGCTCGCGG AGCTGGACCG
47101 GGCGCTGAAC CAGCAGCTGC ACGGCGGCGG GGGCGACGCG GCGGTGGCCG ACATCGACTG
47161 GGAGCGGTTC CTCGCGTCGT TCACCTCCGT ACGTCCCAGC GTTCTCTTCG ACGAGCTGCC
47221 CGAGGTACGC CGTCTCCGCG AGGCGGAGGC GGCGGCCATG GCGGACCAGG CCGCCGCCCG
47281 GACGGGAGCG CCCGGCGGAA CGGAGCTGGC GCGCTCTCTG CGGGCCAAGT CCCTGAACGC
47341 CCAGCGAACT GCGCTCCTGG AATTGGTCAC TGCCCACGTG GCGGCCGTGC TGGGAGAGAG
47401 CGTTCCCGAG GCGATCGACC GGAGCCGGGC GTTCAAGGAC ATCGGCTTCA CCTCCATGAC
47461 CGCGATGGAA CTGCGCAACC GGCTCAAGGA GGCCACCGGG CTCGCCCTTC CTGCCTCCCT
47521 CGTCTTCGAC CACCCCCACC CCGGCGCACT CGCCGACCAC CTGCGCGAGG AACTCCTGGG
47581 CGAGGACGGT GCGGCGGGCG CCGACTCCGC GGCGGAGGAA CCGAGCGCTA CCTCTCCGAC
47641 GGTCCAGGAC GAGCCGATCG CCATCATCGG CATGGCCTGC CGCCTCCCTG GTGACGTCGG
47701 AACACCCGAC GAACTCTGGG AGCTGCTGGA AACCGGCCGC GACGCGATGT CGGACCTGCC
47761 CGTCAACCGC GGGTGGGACG TGGCGGGGCT CTACGACCCG GATCCAGACG CGGCGGGGCG
47821 TTCCTACGTC CGGGAGGGCG GGTTCCTCCA CGACGCGGGG GAGTTCGACG CGGAGTTCTT
47881 CGGCATCTCG CCGCGTGAGG CGCTGGCGAT GGACCCGCAG CAGCGCATCG TCCTCGAACT
47941 CGCCTGGGAA TCGTTCGAAC GTGCGGGCCT GGACCCGGCC GGCCGCCGCG GCAGCCGTAC
48001 CGGCGTGTTC ATGGGAACCA ACGGCCAGCA CTACATGCCG CTGCTGCAGA ACGGCAACGA
48061 CAGCTTCGAC GGCTACCTCG GCACCGGGAA CTCGGCCAGT GTCATGTCGG GCCGCATCTC
48121 GTACACCCTC GGCTTGGAGG GGCCGGCGCT GACGGTGGAC ACGGCGTGTT CGTCGTCGCT
48181 GGTCGCGCTG CATCTGGCGG TGCGGGCGCT GCGCAACGGT GAGTGCGACC TCGCCCTCGC
48241 CGGCGGCGCG ACCGTGATGT CGACGCCGGA AGTCCTGGTG GAGTTCTCCC GGCAGCGTGC
48301 AGTCTCCGCA GACGGTCGCT GCAAGGCGTT CTCCGCCTCG GCCGACGGCT TCGGACCGGC
48361 CGAGGGTGCG GGCGTGTTGC TCGTCGAGCG GTTGTCGGAT GCGGTGCGTC ATGGGCGTCG
48421 GGTGTTGGCG GTCGTGCGTG GTTCGGCGGT GAATCAGGAC GGTGCGAGTA ATGGGTTGAC
48481 GGCTCCGAAC GGTCCGTCGC AGCAGCGGGT GATTCGTCAG GCGTTGGCGG ATGCGCGGTT
48541 GGGTGTGGCT GATGTGGATG TGGTGGAGGG GCATGGGACG GGGACGCGTC TGGGTGATCC
48601 GATCGAGGCG CAGGCGTTGT TGGCGACGTA TGGGCAGCGG GATGCGGGTC GGCCGTTGCG
48661 GCTTGGTTCG TTGAAGTCGA ACGTGGGGCA TACGCAGGCG GCTGCCGGTG TGGCGGGCGT
48721 GATCAAGATG GTCATGGCGA TGCGGCACGG TGTCCTGCCG AAGACGCTGC ACGTCGATGA
48781 GGTCTCTCCG CACGTCGACT GGTCGGCGGG TGCGGTGTCC CTGCTGACGG AGCAGGAGCC
48841 GTGGCCGGAG GTGGGGCGCC CTCGCAGGGC TGCGGTCTCT TCGTTCGGGC TCAGCGGGAC
```

48901 GAATGCGCAT GTGGTGGTCG AGGAGGCGCC GGTCGGGGAG GCGGGGCAGG CCGCCGGGGA
48961 TGCTCGGTTG GCTGTGGTGC CGGTGGTGGT GTCGGGCCGG TCTGCGGGTG CGGTTGCTGA
49021 ACTGGCCTCC CGCTTGAACG AGTCGATTCG TTCGGATCGG TTGGTGGATG TGGGGTTGTC
49081 GTCGGTGGTG TCGCGGTCGG TGTTCGAGCA CCGGTCCGTG CTACTGGCGG GGGACTCTGG
49141 CGAGCTGCAT ACCGGTCTGG TTGCTGTCGG GACTGGTGTG CCGTCGCCTG GTGTGGTGTC
49201 GGGTGTGGCG TCGGTCGGGG GTGGCCGGTC GGTGTTCGTG TTCCCTGGTC AGGGGACGCA
49261 GTGGGCGGGG ATGGCGCTCG GGTTGTGGGC GGAGTCGTCG GTGTTCGCGG ACTCGATGGC
49321 GCGGTGTGAG GCGGCGTTCG AGGGGTTGGT GGACTGGAGT CTGGCGGATG TGCTGGGTGA
49381 CGGGTCCGCG TTGGAGCGGG TCGATGTGGT GCAGCCGGCG TCGTTCGCGG TGATGGTGTC
49441 GCTTGCTGAG CTGTGGCGGT CGTTGGGTGT GGTGCCGGAT GCGGTGGTGG GGCATTCGCA
49501 GGGGGAGATC GCTGCTGCGG TGGTGGCGGG TGGTCTGTCG TTGGAAGACG GGGCGCGTGT
49561 GGTGGTGTTG CGTGCGCGGT TGATCGGCCG TGAGCTGGCC GGGCGCGGTG GGATGGCGTC
49621 GGTGGCGCTG CCGGTCGCGG TGGTGGAGGA GCGTCTGGCG GGGTGGGCGG GGCGTCTGGG
49681 TGTGGCGGTG GTCAACGGAC CGTCCGCCAC GGTCGTCGCG GGTGATGTGG ATGCGGTGGC
49741 GGAGTTTGTG ACCGCGTGCG AGGTGGAGGG GGTTCGGGCG CGTGTTCTGC CGGTGGACTA
49801 CGCCTCGCAC TCGGCGCACG TGGAGGACCT GAAGGCCGAG CTTGAAGAGG TGCTGGCCGG
49861 CATCGGCCCG GTGACCGGTG GGATCCCGTT CTATTCGACG TCCGAAGCCG CGCAGATCGA
49921 CACGGCTGGT CTGGACGCGG GGTACTGGTT CGGGAATCTG CGTCGGCCGG TGCGGTTCCA
49981 GGAGACGGTC GAGCGGTTGT TGGCGGATGG TTTCCGGGTG TTCGTGGAGT GTGGTGCGCA
50041 TCCGGTGCTG ACGGGGGCGG TGCAGGAGAC CGCGGAATCC ACCGGTCGCC AGGTGTGTGC
50101 GGTCGGATCC CTGCGTCGTG ACGAGGGAGG TCTGCGCCGC TTCCTCACCT CTGCGGCGGA
50161 GGCGTTCGTC CAGGGGGTCG GGGTGTTCTG GCCGGCACTG TTCGACGGCA CCGGCGCCCG
50221 TATCGTCGAC CTGCCCACCT ACCCCTTCCA ACGACGGCAC TACTGGTACA ACGACCCTGC
50281 CCGCCGCACG GGCGATGCCA CCTCCTTCGG TATGGCGCAG GCCGGTCATC CCTTGCTCGA
50341 TGCCGGTACG GAGCTCCCTG AATCAGGCGA GCACCTCTAC ACCGCTCGGC TCGCCGCCGA
50401 CTCGCATCCT TGGCTGCTGG AACACACCCT GCTGGGTGCG CCGTTGCTGC CCGGTGCGGC
50461 GTTCGTCGAC CTCGTCCTGT GGGCCGGCGG GGAGGTCGGA TGCGACCTGA TCGAAGAGCT
50521 GACGCTGACC TCGCCGCTGC TGTTGTCCGA CAGCGCTGCC CTTCAACTGC GGCTGGTCGT
50581 GGGCACGGCG GACGCCGAGG GACGTCGTAC GATCACCGTC CACTCGCGGC CGGACGGAGA
50641 CCCGCGTACG ACGCGCACAC CTGCGGCATC GTCCGAGACC AGCCCGGACG CGGAGTCGGA
50701 CACGGAGATC CGTAGGGACA CGTCCGCCTG GACGAAGCAC GCTCAGGCGA CGGTCGCCCC
50761 CGCCCCTGAC GTCCCCCCTT CCGGGGTGGA CGCGGAAGGG GACGCCGTTC GCCCCGCAGT
50821 GGAATGGAGC GTGGCGGCGA CGGAGTCGGA TGCCTTCCAG GCCGAGGACT TCTACGCGTC
50881 CTTCGCCGCA CACGGCTATG GCTACGGCCC GCTGTTCCAG GGCGTACGGT CAGGCCGTCA
50941 GGACGGGACC GACGTCTACG CCGAAGTCGC CCTGGATCAC GACCGCTTGC CGTCTGCCGA
51001 GCAGTTCGGC CTGCACCCCG CGCTGCTCGA CGCGGCGTTC CAGACGATGC GTCTGGGATC
51061 GTTCTTCCCC GACGACGGAC AGGCACGTGT GCCGTACACC TTCCGGGGGA TTCGTCTCTA
51121 CGCCCCGGGA GCCGCGCGCC TGCGGGTCCG TGTCTCGGCG GTCGGGGCCG ATGCCGTACG
51181 CGTGGAGTGC GCCGATGAGC GGGGGCGGCT CGTCTGTGAG ATCGACGCCC TCGTCGTCAG
51241 CACGGTCTCC CCGGACCAGT TGCGGCCGGC CGGACAGGAC GCGACCCAGG ACATGCTGCA
51301 CCGGATCGAG TGGCCCGTCC TCTCCCCGCC GACCGGCAGC GCCACCTCCC CTGCTCCGCC
51361 CCGCTGGATC GTGGTCGGGG GCGAGGACGA GGGCCTCGGG CTCGGGGGCC TTCGACTCGA
51421 CGGTCCGAGG CTTGACGGTC CCGGGCTTGC GGAAGCGCTG TCCGAAGCCG GTATGGGGAC
51481 CGAGCGTCAC CGGAACCTGG CCGACGCGCT GTCGGCCGTA CGGACGCCGG TGGACACGGC
51541 AGGCTCCGCT GCCGCCGCCG GCACGACCTC CCTCATAGCC GTCCCCGTAC CGCAGTCGCC
51601 CACCATGGAC GCCGGTGCCG TGCGCCACGC CGTCCACCGA GCCCTGGAGC TGGTGCAGGG
51661 CTGGGTGGCG GCGGACGAGG CGGCGGAAGA GGGCGGGAGC GACGGTGCCG CGGCCGACCG
51721 GCGGCTGGTG CTGGTCACGA GCGGAGCGGT GTCCACGGGT GACGCCGACC CGCTGCGCGA
51781 CCCGGTGGCC GCGGCCGTCT GGGGTCTGAT CAAGTCCGCC CAGTCGGAGC AGCCCGGCCG
51841 CATCGTCCTC GTTGACCTCG ACGAGGGAGC CGTGGACGGG GCGGCCTTGG CAGCCGCGAT
51901 CTCGACCGGC GAACCACAAC TCGCCCTGCG CGACGGCGAT GTGCACGTGC CCAGGCTGGC
51961 ACCCCTGTCC GTGCGGGACT CGCAGACGCT GCTGCCGCCC GCCGGTACGC GCGCCTGGCA
52021 TCTGGTCGGC GCCGGCACCG GAACCCTTTC GGACCTCGCG CTCGTACCGG CGCAGACCGA
52081 CACCGTCGCG CTCGCACCTG GGCAGGTGCG GATCGCGGTG CGAGCCGCCG GACTCAACTT
52141 CCGGGACACG CTCATCGCGC TCGGTATGTA TCCGGGCGAG GGCGTGATGG GCGCCGAGGG
52201 CGCCGGAGTG ATCACCGAGG TCGGCCCGGA CGTGGTGAGC CTCGCCGTCG GGGACCGCGT
52261 CCTGGGCATG TGGACCGACG GGTTCGGGCC GTACGTCGTG GCCGACCACC GCATGGTGGC

52321 CCCGATGCCG CGCGACTGGT CCTACGCGGA GGCCGCTTCG GTACCCGCCG TCTTCCTCAG
52381 TGCCTACTAC GGACTCAGGC ACCTGGCCGG TCTGCGCGCC GGCCAGTCGG TGCTGGTGCA
52441 CGCGGCGGCG GGCGGTGTGG GCATGGCTGC CGTCCAACTC GCCCGGCACT TCGGGGCCGA
52501 GGTCTTCGGC ACGGCCGGCA CGGCCAAATG GGACGCACTG CGGGCACAGG GCCTGGACGA
52561 CCGGCACATC GCCGGTTCAC GGACGCTGGA CTTCGCGGAC CGGTTTCTCG ACGCGACCGA
52621 GGGGCGCGGC GTGGACGTCG TCCTGAACTC GCTGGCGGGC GACTTCGTCG ACGCCTCCCT
52681 GCGACTGCTG CCGCGCGGAG GGCGGTTCGT GGAACTGGGC AACCCCGACG TACGCGACGC
52741 CGCGCAGGTC GCCGCCGACC GGCCGGGAAC CGTCTACCGG GCCTTCGAGC TGATGGAGGC
52801 CGGGCCGGAG CTGATCGGCC GCATGCTGAA CGAACTGCTG GAACTGTTCG AGTCCGGGGC
52861 GCTGCGCCTG CTGCCCGTCA CCCCGTACGA CATCCGGCGG GCACCCGACG CCTTCCGCAC
52921 GCTCAGCCAG GCCGGTCACG TCGGCAAACT GGTCCTGACG ATGCCACCGG CCTTCGAACC
52981 CCACGGCACG GTCCTGATCA CCGGCGGCAC CGGGAACCTG GGCGGAACAC TCGCCCGCCA
53041 TCTCGTGACC GAACACGGAG TGCGCCACCT GCTCCTGGCC GGACGTAGGG GGCCCGAGGC
53101 CGAAGGCGCC GCCGAACTCG TACGGGAACT GCACGACCTG GGCGCCTCCG TCACGGTCGC
53161 CGCCTGTGAC GTGGCCGACC GAGCGGCGCT CCGGAAACTC CTCGGCGGCA TACCGCCGGA
53221 GCGCCCGCTC ACCGGAGTCG TCCACGCGGC GGGCGTTCTC GACGACGGCG TGGTCACGTC
53281 ATTGACCCCG GACAGGGTCG ACGGCGTCCT GCGGCCCAAG GTGGATGCCG CTCTCAACCT
53341 CCACGAAGCG GCCCTCGATC CCGAACTCGG TCTCGACATC ACCGCGTTCG TCCTGTTCTC
53401 GTCCGTCGCT GCCCTGCTCG GCGGCTCGGG TCAGGGAAGC TACGCCGCCG CCAACGGCTT
53461 CCTCGACGGA CTCGCCCAGT ACCGGCGTGG CCGCTCGCTG CCCGCGCTCT CCCTCGGCTG
53521 GGGCCTGGCC GGGAGCGGCC GGATGACATC CCACCTGGAC AGCCGGGCCC TGCTCCGGCG
53581 CATGGCCAGG GGCGGTGTCC TGCCGCTGTC CCCGGCGGAG AGCATGGCAC TGTTCGACGC
53641 GGCCCAGGGC TTCGACGAGG CGCTCCAGGT GCCGGCGCGC TTCCACACCG CCGCACTGGG
53701 CGCCGACGGC AACGTCCCGC CGCTCTTCAA CGGACTGATC CGGGGCGGGA CGGCGCATGC
53761 CGAGGCCCGG CGCAGGACGG TCGGCGCGTC GCCCGCGGGT GGTCCTGCCG GAGGCGAGCC
53821 GGTGAACCTC GCCGACCGGC TGTCCGGACT GACGGAGGAC GAACAGCGGG CGCTGCTCCT
53881 CGACACGGTG CGCACGCACG CGGCTCTCGT CCTGGGCCAC ACGGGCACGG ACGGCATCCA
53941 GGCCGACCGG GCGTTCAAGG ACCTGGGATT CGACTCGCTG ACGGCCGTCG AGATGCGCAA
54001 CCGGCTCACC GCCGCCACGG GGCTGCACCT CGCGGCGACG CTGGTCTTCG ACCACCCCGC
54061 TCCGGCGGAC CTCGCCGAGC ACCTCCGCTC TCGACTCGTC CCCGAGGGGA CGGACGTACC
54121 GCCGCTCCTC GCGGAACTCG GTCGGCTCGA AACGGCGTTC AAGAAGCTGA CCACCGCGGA
54181 CCTCGCCTCG GTCGTGCCCG ACGACATCGC CCGCGACGAG ATCGCCGTAC GTCTCGCCGC
54241 CCTCGGTTCC CTGTGGAACG GGCTCCATGG CAACGGCCTC AGCGGAGACG CGGCGCAGAA
54301 GCACGGCGAC TCGATCGTCG AGGACATCGA CTCCGCCGAC GACGACGAGA TCTTCGCCTT
54361 CCTCGACGAG AGCTTCGGCG ACTCCTGACC GCAGGCACCT CCGTACGGAC CGACGACTCT
54421 GCAGACGGGT GATGAGAGAT GGCGACCGAA CACGAGCAGA AGCTCCGCGA CTACCTCAAG
54481 CGGGCCACCA CCGAACTCCA CAAGGCCACG GAACGGCTGA AGGAGGTCGA ACAACGCGCT
54541 CACGAGCCGG TTGCGATCGT GGGGATGGGA TGCCGGTTCC CGGGCGGGGC GTCCTCGCCT
54601 GAGGAGTTGT GGGACCTGGT GGCGGCGGAG ACGGACGCGG TCTCCCCCTT CCCGGTGGAC
54661 CGGGGGTGGG ACGTGACGGG GCTGTACGAC CCGGATCCGG ACGCGGCAGG GCGTGCCTAC
54721 GTCCGCGAGG GCGGGTTCCT CCACGACGCG GGGGAGTTCG ACGCGGGGTT CTTCGGAATC
54781 TCTCCGCGTG AGGCGTTGGC GATGGATCCG CAGCAGCGGT TGCTGCTGGA GACGTCGTGG
54841 GAGGCGTTGG AGCGGGCGGG CATCGATCCG CACACGCTGC GCGGCACGCG GACCGGGGTC
54901 TACATGGGTG CCTGGAACGG CGGATACGCC GAGGGGATTC CCCAACCCAC GGCGGAACTG
54961 GAGGCCCAGC TCCTCACCGG CGGCGTGGTG AGCTTCACCT CGGGCCGTGT GTCCTACCTC
55021 CTGGGTCTGG AGGGGCCGGC GGTGACGGTG GACACGGCGT GTTCGTCGTC GCTGGTCGCG
55081 CTGCACCTGG CGGTGCGGGC GCTGCGCAGT GGTGAGTGCG ACCTCGCCCT CGCCGGCGGC
55141 GCGACGGTGA TGTCGACGCC CGACGTGTTC GTGCGCTTCT CCCGGCAGCG AGGAGTGGCC
55201 GCGGACGGTC GCTGCAAGGC GTTCTCCGCG TCGGCCGACG GATTCGGACC GGCTGAGGGT
55261 GTGGGCGTGC TGGCGGTGGA GCGGTTGTCG GATGCGGTGC GTCATGGGCG TCGGGTGCTG
55321 GCGGTCGTGC GTGGTTCCGC GGTCAACCAG GACGGTGCGA GCAACGGACT GACGGCGCCG
55381 AGCGGACGAG CTCAGGCCCT TCTGATTCGT CGAGCGTTGG CGGATGCGCG GTTGGGTGTG
55441 GCTGATGTGG ATGTGGTGGA GGGGCATGGG ACGGGGACGC GTCTGGGTGA TCCGATCGAG
55501 GCGCAGGCGT TGTTGGCGAC GTATGGGCAG CGGGATGCGG GTCGGCCGTT GCGGCTTGGT
55561 TCGTTGAAGT CGAATGTGGG GCATACGCAG GCGGCTGCCG GTGTGGCGGG CGTGATCAAG
55621 ATGGTCATGG CGATGCGGCA CGGTGTCCTG CCGAAGACGC TGCACGTGGA TGAGCCGACG
55681 GCGGAGGTGG ACTGGTCGGC CGGCGCGGTG TCTTTGCTGA GGGAGCAGGA GGCGTGGCCT

55741 GAGGTGGGGC GTCTGCGTAG GGCTGCGGTG TCTTCGTTCG GTGTGAGTGG GACGAACGCG
55801 CATGTGGTGG TGGAGGAGGC GCCGGTTCCG GAGGACGGGG AGGCGGTCGG GGGCGGTGTG
55861 CCTTTGGCTG TGGTGCCGGT GGTGGTGTCG GGTCGTTCTG CGGGTGCGGT GGCGGAGCTG
55921 GCGGGCCGGG TCAGCGAGGT GGCTGCGTCT GGTCGGTTGG TGGATGTGGG GTTGTCGTCG
55981 GTGGTGTCGC GGTCGGTGTT CGAGCACCGG TCCGTGGTAC TGGCGGGGGA CTCTGCCGAG
56041 CTGAATGCCG GTTTGGATGC TGTGGCCGGT GGTGTGCCGT CGCCTGGTGT GGTGTCGGGT
56101 GTGGCGTCGG GTGAGGGTGG CCGGTCGGTG TTCGTGTTCC CTGGTCACCC GACGCAGTGG
56161 GCGGGGATGG CGCTCGGGTT GTGGGCGGAG TCGTCGGTGT TCGCGGAGTC GATGGCGCGG
56221 TGTGAGGCGG CGTTCGTCGG CTTGGTGGAC TGGCGCTTGT CGCAGGTTTT GAGCGATGGG
56281 TCGGCGCTGG AGCGGGTGGA GGTGGTGCAG CCGGCGTCGT TCGCGGTGAT GGTGTCGCTT
56341 GCTGAGCTGT GGCGGTCGTT GGGTGTGGTG CCGGATGCGG TGGTGGGGCA TTCGCAGGGG
56401 GAGATCGCTG CTGCGGTGGT GGCGGGTGGT TTGTCGCTGG AGGACGGGGC GCGTGTGGTG
56461 GTGTTGCGTG CGCGGTTGAT CGGTCGTGAG CTGGCCGGGC GCGGTGGGAT GGCGTCGGTG
56521 GCGCTGCCGG TCGCGGTGGT GGAGGAGCGT CTGGCGGGGT GGGCGGGGCG TCTGGGTGTG
56581 GCGGTGGTCA ACGGACCGTC CGCCACGGTC GTCGCGGGTG ATGTGGATGC GGTGGCGGAG
56641 TTCGTGGCCG CGTGCGAGGT GGAGGGGGTT CGGGCGCGTG TTCTGCCGGT GGACTACGCC
56701 TCGCACTCGG CGCACGTGGA GGACCTGAAA GCCGAGCTTG AACAGATTCT GGCCGGCATC
56761 GGCCCGGTGA CCGGTGGGAT CCCGTTCTAT TCGACGTCCG AAGCCGCGCA GATCGACACG
56821 GCTGGTCTGG ACGCGGGGTA CTGGTTCGGG AATCTGCGTC GGCCGGTGCG GTTCCAGGAG
56881 ACGGTCGAGC GGTTGTTGGC GGATGGTTTC CGGGTGTTCG TGGAGTGTGG CGCGCATCCG
56941 GTGCTGACGG GGGCGGTGCA GGAGACCGCG GAATCCACCG GTCGCCAGGT GTGTGCGGTC
57001 GGATCCCTGC GTCGTGACGA GGGAGGTCTG CGCCGCTTCC TCACCTCGGC CGCGGAGGCA
57061 TTCGTCCAAG GCGTGGAGGT GTCCTGGCCG GCACTGTTCG AAGGCACCGG CGCCCGCACG
57121 GTCGACCTGC CCACCTACCC CTTCCAACGT CGGCGCTACT GGCTGGAGTC GCGCCCTCCC
57181 GCGGCGCCGA TCGAGACTGC CGCAGCCTCT GGCATCGAGA GCTGGCGCTA CCGCGTGGCG
57241 TGGAAGAGCC TGTCGCTGTC GGAGTCGTCG CGTCTTGACG GCCGGTGGCT GCTCGTCGTG
57301 CCCGAAACCC TGGACGCCGA CGGCACGCGG ATCGCCCACG ACATCCAGCA CGCCCTCACC
57361 ACCCACGGCG CCACGGTCTC CCGTCTGACG GTCGACGTGA CGACGACCGA CCGCGCCGAC
57421 CTGTCGGCGC GGCTCACCAC CACCGCGGCC GAAGACCAGG GGCCTCTCCG GGGCGTCCTC
57481 TCCCTCCTGT CCACCGATGA ACGGCAGCAC CCGGATCATC CCGGTGTCGA CCGTGCCACG
57541 GCGGGCACGA TGCTGCTCGC CCAGGCGTGC GGGGATCTGG TCGTGGCCCG GGGCGTGGAG
57601 CCGAGGCTGT GGGTCGTGAC CCGCGGGGCG GTCGCGGTGT CCCCCGCCGA GCGTCCGTCG
57661 TCAGCCGGCG CCCAGGTCTG GGGCCTGGGG CGCTGCGCGG CGCTCGAACT TCCCACTCGG
57721 TGGGGTGGGA TGGTCGACCT TCCCCCGGCG GCCCGGGATG CTGGAAGGCA CGTACGGCGG
57781 CTCGTGCGTC TGCTGTCGGA GACCTGTGCG GAGGACCAGG TGGCGCTGCG TGCGTCGGGT
57841 GCGTACGGCC GCAGGCTGCT GCCCGCGTCC AGCCCCTCCG TATCCGTCCC CCGGACCGCG
57901 AAGAGCGGCT ACCAGCCGCG CGGCACGGTG CTGGTGACCG GCGGAACCGG TGCCCTCGGT
57961 GGCCACTTGG CACGGTGGCT GGCCCGCAAC GGCGCCGAGC ACATCGTTCT GGCCGGGCGT
58021 CGGGGCGAGG GTGCTCCAGG AGCCGCGGAA CTGTCGGCGG AGCTCAAGGA GCTGGGTGCG
58081 GAGGTCACCG TCGCGGCCTG CGACGTGGCG GACCGGAACG CGTTGCGTGA CATGCTGGAA
58141 TCCCTGCCGG CCGACCGGCC GCTGTCGGGG GTGTTCCACG CTGCCGGTGT CCCGCACTCG
58201 GCGCCGCTGG CCGAGACGGA TGTGGCGGGG CTCGCCGCCG TGCTCCCGGG GAAGGTCGTC
58261 GGGGCACGGC ACCTGCACGA ACTCACCAGG GAGAAGGAAC TGGACGCGTT CGTGCTGTAC
58321 GCGTCGGGCG CCGGGGTGTG GGGGAGCGGC GGGCAGAGCG CGTACGGAGC CGCCAACGCC
58381 GCACTGGACG CGCTGGCCGA ACAGCGCCGG GCTGAGGGAC TGCCCGCCAC TTCGGTCTCC
58441 TGGGGCCTGT GGGACGGCGG AGGCATGGCC GGCGAGCGAG GCGAGGAGTT CCTCACCGCC
58501 CTCGGCCTGC GGGCCATGGA GCCCGAGTCG GCTGTCGCCG CCCTGGAGGA GGCCCTGGAT
58561 CGTGGGGACA CCTGCGTGAG CGTGGTCGAC GTCGACTGGT CCCGGTTCGC CGAGTCGTTC
58621 ACCGCCTTCC GGCCCAGCCC GCTGATCGGG GAGCTCCCCG GGGTACGTGC CGTGCCCGAC
58681 GGATCGGCGG GCGGACCGTC GGACGACCTC GCGGACGCTG CGCGGCACGG CGGGGCAGCC
58741 GACCGGGGTG TGCCTGCAGG GCTCGCCCGG GCGACGGGCG ACGACCGGCA GGACATCCTG
58801 CTCGATCTCG TACGCCGCCA TGCCGCCGCC GTCCTCGGTC ACCCGGGACC GCAGCACATC
58861 GAGCCCGACG CCGGTTTCCG GACCCTGGGG TTCAGTTCGG TCACCGCGGT GGAACTGGCC
58921 AACAAGCTCG GTGCGGCCGT GGGAACGAAG ATCCCCGCCA CCTTCGCGTT CGACCACCCC
58981 AACGCCCGTG CCGCGGCGTC CCGCCTCGAC GTCCTGTTGG CGGCGTCGAG CGATGAGACC
59041 GCGCAGGAGG CGGAGATCCG GCAGGCACTG CGGACTGTGC CGCTGGCCCG GCTGCGGGCT
59101 GCGGGGCTCC TCGACGGCCT GCTCGAACTC GCCGGGCTGG AAGCGGAGCC CGGCCTGCCG

```
59161 GGCGACGTAC CGGATCGCGG TGCGGCCACG CCGGACGAGG AGTCCGCCCT GGCGGAAGTC
59221 GACGGCCTGG ACGCCGAAGC ACTGGTCGAC CTCGTCCTCA ACCAGTCCGA CTCCTGACCG
59281 CCGGCGGCGG CGCCGCGGCC CGCCGTGCCG TCGCCGCCCT CGGCCGTACG AAGAACCCCA
59341 CAGACCTGAC CGGGTCACGG CCCGGTGCTC AGCAAGGAGA CCACTCATGG CTCTGTCCCA
59401 AGAGAAGGTA CTGGAGGCAC TGCGCACCTC CGTCAAGGAC GCCGAACGGC TGCGCAAGCG
59461 CAACCGCGAA CTCCTCGCGG CCCGCCACGA GCCCATCGCC GTCGTCGGCA TGGCCTGCCG
59521 CTATCCCGGC GGGGTCCGTT CGCCCGAGGA CCTCTGGGAA CTCGTCGTCT CGGGCACGGA
59581 CGCGGTGGGT CCCTTTCCCG AGGACCGTGG CTGGGACGTG GAGCGGATCT ACGACCAGGA
59641 CCCGTCCGTC CCGGGCACCA CGTACTGCCG CGAGGGCGGA TTCCTTTACG ATGCGGGGGA
59701 CTTCGACGCG GCTTTCTTCG GGATAGGGCC GCGCGAGGCC ACCGTGATGG ACCCCCAGCA
59761 GCGCCAGCTG CTGGAGGCGT CCTGGGAAGC CCTGGAGCAG GCCGGGCTGG ACCCCCGGGC
59821 GCTCCGCGGC AGCCAGGGGG GTGTGTTCGT CGGCGCGGCG AACCAGGGCT ACGTACCGGG
59881 TGACGCGGCG GCGTCGGGAC GTCTGCCGGA GGGTTCCGAC GGCTATCTGC TCACCGGCAA
59941 CGCCGACGCC GTCCTGTCGG GCCGGATCAG CTACTTCCTG GGCCTGGAAG GCCCGTCCAT
60001 GACCGTCGAG ACGGCCTGCT CCTCCTCCCT GGTGGCACTG CACCTGGCGG TGCAGGCGCT
60061 GCGCCGTGAG GAGTGCGAGT TCGCCCTGGC CGGAGGGGTC GCCGTGCTCG CCAACCCGGC
60121 CGCCTACGTG GAGTTCGCCC GGCAGCGGGG ACTCGCCCCG GACGGGCGCT GCAAGGCGTT
60181 CGACGACGCG GCGGACGGTA CGGGCTGGGC CGAGGGCGTC GGCGTCCTGG TGGTGGAGCG
60241 GCTGTCGGAC GCGGTACGCA AGGGGCACCG GGTCCTCGCC GTCGTGCGGG GCACGGCGGT
60301 GAACCAGGAC GGTGCCAGCA GCGGTCTGTC CGTGCCCAAC GGGCCCTCCC AGCAGCGGGT
60361 CATCCGCCGA GCGCTGGCCG ACGCCCGGCT GGAGGCCGGC CAGATCGACG CGGTGGAGGC
60421 CCACGGCACC GGCACTCGGC TGGGGGACCC CATCGAGGCG CAAGCCCTGC TGGACACGTA
60481 CGGAGAGGAG CGGAGCCCCG AACGCCCTCT GTGGGTCGGG TCGTTGAAGT CGAACTTCGG
60541 TCACGCACAG GCGGCAGCCG GAGTCGGCGG CGTCATCAAG ACGGTGATGG CGCTCCGGCA
60601 CGGCCTGCTT CCCCGCACGC TCCATGTGAC CAGCCCGACG CGGCACGTCG ACTGGGGCGA
60661 CGGACAGGTG CGGCTGCTGA CCGAGCCGGT CGACTGGCCG CGGACCGGCG CCCCCCGGCG
60721 GGCCGCGGTC TCGGCGTTCG GCGTGAGCGG CACCAACGGG CACATCATCC TCGAGGAGGC
60781 GCCGCCGCCC ACCCGGCCCG AAGCGGTCCG GCAGGCCGGG GAGCGGCGGC CGGTCCTGGT
60841 CCCGTGGACG CTGTCCGGCC GTACGAGGCC GGCGCTGTGC CGGCAGGCCG CGCGCCTGGC
60901 GGCGCACCTC GAACAGCACC CGGACCTCGA CCCGCTGGAC GTCGGGTTCT CGCTCGCCAC
60961 GACGCGCACC CACTTCGAGC ACCGGGCCGT GCTGCTCGCG GACGCCGCCA CCGAGGGCGG
61021 CTCCCGTGCC GACGCGCTCG GGGCGTTGCG GGCGATCGCG GAGGACCGCG ACCCGGGCGG
61081 GGCGGTACGG GACACCGCGC GGGGCGAAGG GCGTATCGCC TTCCTGTTCT GCGGGCAGGG
61141 CAGCCAGCGG CCCGGCATGG CGGAGCAGCT GTACGCGCAG TACCCGGCGT TCGCGCGGGA
61201 ACTGGACACG ATCGCGACGC ATCTGGACGC CCATCTGGAC CGTCCGTTGG CGACGGTGAT
61261 GTTCGCGCCG GCCGGTACGG CGGAGGCCGC GCTGCTCGAC GGCACGCAGT ACGCCCAGGC
61321 GGCCCTGTTC GCCGTAGAGG TCGCGTTGTT CCGGCTCTTC GAGGGCTGGG GGCTGCGCCC
61381 CGACGTACTG CTGGGCCATT CCGTGGGCGA GCTGGCCGCC GCCCACGTGG CCGGGGTGTT
61441 CGGGCCGGCG GACGCCTGCT CGCTGGTCGC CGCACGCGGC CGGCTCATGC AGGAGCTGCC
61501 GGCCGGCGGC GCGATGCTCT CGGTCCGTGC CGCCGAGCAC GAGGTGCGGG AGCTGATCGC
61561 CGGGCAGGAG GACCGCATCG CGGTGGCGGC CGTCAACGGG CCCCGCTCCG TGGTCGTATC
61621 CGGGGACGAG GACGCGGTCT CGGCGCTCGC CGAGGAGCTG ACCGAATACG GCGTGCGCAC
61681 CAAGCGCCTC AACGTCAGCC ACGCCTTCCA CTCCCCACGT CTGGACTCCA TGCTGGAGAC
61741 GTTCCGCCGG GTCGCGGAGA CGGTGGAGTA CCGCGAGCCG ACGCTCGACG TGATCAGCGG
61801 CCTGACCGGC CGCCCGGCCG ACGCCGGGGA ACTCGCCACC GCCGACTACT GGGTCCGGCA
61861 GGCGAGGGAG ACCGTCCGGT TCCACGACGG GGTGCGCGCC GCGCACGCGC GCGGCGTCAG
61921 CACCTTCGTG GAGCTGGGGC CGGACGGCGT GCTGTGCGGC CTGGCCCTGG AGACCCTGGC
61981 GGAGGAGACC GACGGGGAAG CGGCCGCCGA GACGCCCGGC CGGGCGCGGG CGGCGCTGGT
62041 GCCCGTGATG CGTCGGGAGC GGCCGGAGGG CAGTACCCTC CTGACGGCGC TCGCCACGGC
62101 CCACGCGCGC GGGGCGGAGG TGGACTGGTC CCGGTTCTAC GCCGACACCG GCGCCCGCCA
62161 CACCACACTG CCCACCTATG CCTTCCAGCG CCAGCGGTTC TGGCTGGAGA CGGCGGCCCC
62221 CGCCGCGCCC GCGGCGGGCC AGGGGGCCGG ACCGGCCGAC CCGCAGGACA GCACCGGTCC
62281 GGCCGCCCGG CCCACGCTGA CGGAACAGGA CCTCCTCCTG CTCGTGCGGA CGGAAGCGGC
62341 GGCCGCACTC GGCCACGCCG AACTGGAGGA CGTACCGGCC GACAGCCTCT TCGGCGACAT
62401 CGGCTTCGAC TCGCTCGCGG CCATCGAACT GGGCGCCGCC CTGACCGGCG CCACCGGGCT
62461 GGAAGTGCCG TCGTCCCTCG TCCTCGACCA CCCCACGCCC AGGGAGCTGG CCGCGCACCT
62521 GGCAGCCGCC CGGACGGCCG CCGACAGCGA CGACACGTCC CCCGAAGGCC CGGACACGGC
```

62581 CGGTGAGAGC AGCCTGTCGG CGATGTACCG GCGGGCCGTG CGGCTCGGCC GGGCCGAGCC
62641 GTTCATCGGC ACACTCGCCG AACTCGCCGC CTTCCGGCCC GTCTTCCCCG CCGATCACAC
62701 CCTCGCGGAC GGCGAGACCG TCGGACAGGC GGCCGCCGCC TGGCAGCCGG CTCCGGTGCG
62761 CCTGGCCACC ACGGACGGTG AGGGACCGGA GCTGATCTGC TGCGCGGGTA CGGCGGTGGC
62821 GTCGGGACCG GAGGAGTTCA CCGCGCTGGC CGCGGCCCTG GGCGACCGGC TGACCGTGTC
62881 GGCACTGCGC CAGCCCGGCT TCCGCGCGAA CGAGTTGCTG CCCGGCTCCC TGGACGGGCT
62941 GCTCGACGCG CAGGCGGACG CGGTGCTGCG GCACACGGGT GACACGCCCT ACGCCCTCCT
63001 CGGCCACTCG GCGGGCGGGG CGCTGGCGCA CGCGCTGGCC TGCCGACTGG AGGAGCTCGG
63061 CGCGGGTCCC GCGGCGCTGG TCCTGGCCGA CGTCTATCTG CCCAGCTCGC CGGGGGCGAT
63121 GGGGGTGTGG CGCAACGAGA TGCTCGACTG GGTCATGCGG CGTTCCGTGG TGTCCATCGA
63181 CGATGCCCGG TTGACGGCCA TGGGCGCCTA CAACCAGATG CTCCTGGAGT GGACACCGCG
63241 GCCCACGAAG GCGCCGGTCC TGTTCCTGCG CGCGACGGAG CCGGTGAGGC CGTGGTCCGG
63301 AGAACCGGAG AGCTGGCGGG CGCACTGGGA CGGCGGCGAC CACACCGCCG TCGACGTGCC
63361 CGGCACCCAC CTGACGCTGA TGACCGAGCA CGCCCGCCAC CTCGCGGCGA CCCTCCACAC
63421 CTGGCTCGGC ACCCTGTGAA CCACGCCCGG GGCGGCTTCG CCGCGCGTAG GACTGCCGCC
63481 TCCCCCGACT TCCGTACACC GCGACACCTT GGAGGACTCC CGTGACAACG CAGTGGACCA
63541 CCCCGTCCGT GCTCGGCCGC AGACTGCAAC GCACCTACGT GGGGCACTGG TTCGCAGGAA
63601 CGCAGGGAGA CCCCTACGCG CTGATCCTGC GCGCCCAGCG GGACGACACC ACCCCCTACG
63661 AGGAGGACGT CCGCGCACGC GGACCGGTGT TCCACAGCGA GGTGCTCGAC ACCTGGGTGA
63721 TCACGGACGG CGCTCTCGCC CGGTCCGTCC TGACCGACGC CCGCTTCGGC GGGCTGACGC
63781 GCGCGGGAGG ACGGTATCGC GCGGAGCTTC TCCCTCCGGC GGGCCCCGAG GTCGGTCCGG
63841 CCCGCGCAGG GGTACGCGGC GGCGTGCGGG CCGACGCCGA TCCGGCGGTG TCGGCGCAGG
63901 ACGAGGTGGT GGTGGAGGCC CTCGCCGAGC AGCTCTCACG CACCCTCCTG GGCGGACTCG
63961 GCGACGACTT CGACCTCGTC GCCGCCTTTG CGCGACGCCT GCCGGCACAG GTCCTGGCGG
64021 AATTCCTCGG GCTGCCCGCA GCCGCGCGCA GCCGGTTCGA GGAACTGCTG GCCGGCTGCG
64081 CCCACAGCCT CGACAGCCGG CTCTGTCCGC AGACGCTCGA CATCACACGG ACCGGCCTCG
64141 GAGCGGCGGC CGAGCTCCGG GAACTGCTCG CGCGCCACCT CGGCGGGAGC GGACCACGCT
64201 CCGCTCAAGC GGCAGTCTCC CTGGCAGTCG AGGTGGCCGC ACCCGCCGGC GCGCTCATCT
64261 GCAACGCGGT CGAGGCGCTG AGCAGCTCTC CCGGGCAGTG GAACGCCCTC CGCCAGAACC
64321 CGGAGAAGGC CGACGCCGTC GTGGCGGAGA CCTGGTGGCG ACGACCGCCG GTGCGGGTGG
64381 AGAGCCGGAT CGCCCAGGAG GACGTCGACG TGGCCGGAGT GCCCGTCCCC GCGGACGGGC
64441 ACGTGGCGAT CCTCGTCGCC GCCGCCCAGC GCGACCCGGC GATCACCCCG GCCCCGACGA
64501 AGGACGACAC CGGCACCCCC GGACAGGGCG ACTGCGGCGT GCCCCTGGGG CTCGTCGGCG
64561 ACGCGCACGC CACCTCCGCC GCCCGGACGG TCCGCGCCCT CTGCCGCGGT GCGCTGCGAG
64621 CGCTCGCGCA GGAGGCACCG GGCCTGCGGC CGAACGGGAC CCCGGTGCGC CTCAGGCGGG
64681 CACCCGTCAC GCTCGGCCAC GCCCGCTTCC CCGTCGCCCG GACGGGCCGG GGGACACCGA
64741 CCGACGCGGG CGCGGCATGA GCACCCGCGA CGACCACCGA CTGCCGAACG GGGAGACGAG
64801 CCGATGCGCG TCCTGATGAC GTCGATCGCC CACAACACGC ACTACTACCA CCTGGTGCCG
64861 CTCGCCTGGG CCCTGAAGGC CGCGGGCCAC GAGGTGCGCG TCGCCGGCCA GCCCCGCGTC
64921 ACGGACATCA TCACCGGGTC CGGACTGACC GCCGTGCCGG TCGGTGACGA CGAGGACATG
64981 ATGGAGCTGT TCGCCGAGAT CGGCGGAGAC ATCACCCCCT ATCAGGAGGG ACTGGACTTC
65041 GCCGAGGAGC GGCCCGAGGC ACGGTCCTGG GAACATCTGC TCGGACAGCA GACCGTTCTG
65101 ACCTCGCTGT GCTTCGCACC GCTCAACGGC GACTCGACGA TGGACGACAT CGTCGCGCTG
65161 GCCCGCTCCT GGCAGCCGGA CCTGGTGATC TGGGAACCCT TCACCTTCGC CGGAGCGGTC
65221 GCCGCCCACG CCGTGGGCGC GGCGCACGCC CGCGTCCTGT GGGGTCCCGA TGTCATCGGC
65281 CGGGCCCGGG AACGGTTCGT GGAGGCCAAG GCACAGCAGG CTCCCGAACA CCGGGAGGAC
65341 CCGATGGCCG AGTGGCTCGG CTGGACCCTG GAGAGGCTGG GCCTCCCGGC CGCCGGAGAC
65401 GGGATGGAGG AGTTGCTGAA CGGCCAGTGG GTCATCGACC CGGGCCCGGA GAGCGTCCGG
65461 CTCGACCTTC GCGAGCCGAT CCTGCCCATG CGTTTCGTTC CCTACAACGG ACCTGCCGTC
65521 GTCCCCGGAT GGCTGTCCGA GAAGCCGAAG CGACCGCGCG TCTGCCTCAC CCAGGGAGTG
65581 TCGGGACGCG AGACCCACGG CAAGGACGCC GTCCGCTTCC AGGACCTGCT CGCGGCGCTC
65641 GGCGACCTCG ACATCGAGAT CGTCGCCACC CTGGACAGCA CCCAGCGGGA GAACCTGACG
65701 GAGGTCCCCG ACAACGTCCG GATCGTCGAC TTCGTCTCGA TGGACGTGCT GCTGCCGAGT
65761 TGCGCCATGA TCATCTACCA CGGTGGCGCC GGCACCTCGG CGACGGCCCT CCTGCACGGC
65821 GTTCCGCAGG TCGTCATCGG AGCGCACTGG GACGTGCCGG TCAGGGCACG GCAGCTCGAC
65881 GACCTGGGCG CCGGCATCTT CATCCGGCCC GAGGACCTCG ACGCCGCCAC ACTGCGCGCG
65941 GCGGTTCAGC GCGTGCTCAC CGAGCCCTCC CTCCAGCGGG CCGCGGACCG GCTGCGGGCC

66001 GAGATGCGCT CCAACCCCAC GCCGGCCGAG ACCGTCACGG TGCTGGAGCG GCTCTCCCGG
66061 AGCCACCGAC AGCCCCGCTG ACCACACGCG GTACACGGTG CGGGCCCACG TGCCGGGGGC
66121 TCCACCGTCG CCGGCGGTCG TCGGATCGCC GTCCGGCCAT GTCCCGGCAC CCAAGGACGG
66181 AGCAGAGCAG AACATGGAAT TCGAAGGTCA GGTCGCGCTC GTCACCGGGG CCGGCAGGGG
66241 GATCGGCCGT GCGACGGTCG TCCGCCTCGC GGAGGCCGGA TGTGACATCG CCCTCCACTA
66301 CAACCAAGCG AAAGCGCAGG CCGAGGAAGT CGCCGAGCGC ATCGCCGCAC TGGGCCGCAC
66361 GGTCGAACTG TTCCCGGGCG ACCTCTCCCG CCCCGAGACC GGGCGACAGC TCGTGCCCGC
66421 GGTGCAGCAG AAGTTCGACC GGATCGACAT CCTGGTGAAC AGCGCGGGCA TCACACGGGA
66481 CAAACTCCTG CTGTCCATGG AGGCGGACGA CATCCACCAG GTCATCGCCA CCAACCTCGT
66541 CGGCCCGATG TTCCTCACCC AGGCGGTCGC GCTCACCATG CTGCGTCAAC GCTCCGGGCG
66601 CATCGTCAAC ATCTCCTCCG CCGCCGCGAG CAGGCCCGGA AAGGGCCAGT CCAACTACGC
66661 CGCGTCCAAG GCCGGTCTGG AGGCCTTCAC CAGGGCCATG GCGGTGGAAC TCGGATCCCG
66721 CGGAATTCTC GTCAACGCGG TCGCTCCCGG CATCGTCAAG ACCGGCCTGA CCGAGGCTCT
66781 CCGCGAGGGG GCGGAGCCCG AACTCCTGGC CCGGCAGGTG ATCGGTTCCT TCGCCGAACC
66841 CGAGGCGGTG GCGGAGGCGG TGGCCTACTT GGCGAGCCCG CGCAACACGC ACACGACGGG
66901 CACGGTCCTC ACCGTCGACG GCGGGCTCAA GATGGTGTGA GGCCCACCGG GCATCGGACA
66961 GCCGGTGGAT CCGCCGGAGG CGGAGACCCG CGCACCGCGG GCGTCGACCC GCGCACCGCG
67021 GGCGCGCGTG GGGGCGCCCG CGGTGCGCGA AGGCCGCCTC TGGCTCGCGT CACCGGAAGA
67081 AGCCCGATTC CCCAGCGGGC GACCGTAGCC GGAGCGTTGG ACCGCCCTGC TCCGCACGTC
67141 GAGCCCCACC GTGGTAGCGG CGGACATGCC CAGGGGGCGC ACGCCCGGAT CCTGCCGCAC
67201 GACCAGGCGC ACCAGGGTTT CGGCGAACAC GGCGGCCGAC GCCGCGGGGT TGCCGCCGCG
67261 GCACGGCCGA CGCGCGCTGG TCGCACGGGG TGAGCCCGCG GGAGCGGAGG GCGCCGGTTC
67321 GCTCAGGACG CCGGGATCTC CGCCGGGACG ACGGACTCGG GGGCGCCGTC GGGGCCGGGC
67381 CTCGTGCCAC CGTCGGCGTC GAGCTGCGGC GGATCGGAGA GCTGCTCGCG CACGTACCCC
67441 CACACCACGG CGACGAGGGC GGCGACGGGC ACGGCGAGGA GGCTGCCGAC GATGCCCGCC
67501 AGACTGCCGC CCAGGGTGAC GGCGAGCAGG ACGACGGCGG CGTGCAGTCC GAGTCCGCGG
67561 CTCTGGATCA TGGGCTGGAA GACGTTCCCC TCCAGCTGCT GCACCACCAC GATGATCGCG
67621 AGCACGATCA GGGCGTCGGT CAGGCCGTTG GAGACCAGGG CGATGAGCAC GGCGACGAAT
67681 CCTGCTAACA GCGCGCCGAT GATCGGCACG AAGGCGCTGA CGAAGGTGAG TACGGCGAGC
67741 GGCAGGACGA GCGGGACGCC GAGGATCCAC AGGCCGATGC CGATGAGGAC GGCGTCGAGG
67801 AGGCCCACCG CGGCCTGGGA GCGGACGAAG GCGCCGAGGG TGTCCCAGCC GCGTTCGGCG
67861 ATCGTGGTGG CGTCGGTCGC GAGGCGGCCG GGGAGCTGAC GGGCGAGCCA GGGCAGGAAG
67921 CGCGGCCCGT CCTTGAGGAA GAAGAACATC AGGAAGAGCG CGAGGACGGC CGTGACGACC
67981 CCGTTCACCA CCGTGCCGAC GCCGGTGGCG AGGGTGGTCA GCAGGGATCC GACGCTGTTC
68041 TGGAGACGGT CGGTCGCGGT GTCCAGGGCG CCGGTGATCT GGTCGTCGCC GATGTTCAGG
68101 GGCGGACCCG CGGTCCACTC GCGGAGACGC TGGATGCCCT CGACGACACC GTCGGCCAGT
68161 TCGCCGGACT GCGAGGCGAC GGGCACGGCG ATGAGCGCGA CGGTGCCGGC GGTGACGGCG
68221 AGGAAGAGGA CGGTGACCAC CGAGGCGGCG AGCGCCGGCG GCCAGCCGAG ACGGCGCAAC
68281 AAGCGGGCGA AGGGCCAGGT CAGCGTGGTG ATCAGCAGAC CGATGACGAG TGGCCACACG
68341 ATCGACCACA TCCGGCCGAG CAGCCAGATC ACCGCCGCCG TGCCCAAGAG CACCAGCAGA
68401 AGCTCCGTCG ATATGCGGGC CGAGGCCCGT AGCGCGGCAC GTGTTCTCGC AGGACTGAGA
68461 GAGGCAGACA TGGCGATCAC CCTAGAGCGG CCCGGGCGGC CCGCTGCCCC CGTGCCCCGA
68521 TCCTTCGCGC CGGGGTGACG CGCATCGGGG GTTCCGGCAC TGCCTGAGCG CCTGCACGGA
68581 CGGGGCGGGT TACGCCGAGG GGAAGCAGCC GGCTCCGGAT CGACAGGAGT GCGGGTGACG
68641 GTCGTACACC GGCGCTACGT CGCCCACTCC GCGGCGCACA AGGGCGGTCG CCGTCTGGCC
68701 CCTCCCCGCG TCCGACGACC GCCCACCCCT CGTCAGGGAG CGGGGTCCGG GGCGAGGTGG
68761 ATACGGGCCG CTACCGGGAG GTGGTCGCTG GCCGTCGCGG GAAGGGTCCA TGCCGAGGCG
68821 GCCCGGACGC CCCCGAGAAG GATCTGGTCG ATCCGGACGA CCGGCAGGCG CGCCGGCCAG
68881 GTGAAGCCGA AGCCCGCGCC CGCCGCGGCC TGTGCGGAGA CGAGACGGTC GGTGAGGGGG
68941 CGCAGTGCGC GGTCGTCCGT GGAGCCGTTG AGGTCGCCCA GGAGGACGAC GCGCCGGACG
69001 GGCTCGGCCC GCACCTCCGC CGCCAGCAGC CCCAGCGCCT CGTCGCGCGC CCCGGCGGTG
69061 AATCCGCCCG GGCCCACGCG GACGGACGGC AGATGGGCGA CATAGACGGC CAGCGGCCCG
69121 CCGGGCGCGT CCACCGTGGC CCGCATCGCC CGCGTCCACG GCATGATCGG CACAGCCCGC
69181 GCGTCGCTCA GCGGATGCAC GCTCCACAAG CCCACCGTGC CCTCGTAGAA GTGGTACGGG
69241 TACGACTCCG CAAGGGCCCG CTCGTAGGCG GGCGCCGTGG CCGGGCTCAG CTCCTCCAAC
69301 GCCAGTACGT CCGCCCCGGC GGCGAGCAGG CTCCGCACCG TTCCGGCGGG GTCGGGGTTG
69361 GCCTGCTCGA CGTTGTGACT GACCAGCGTG AGGTCCCCAC CGGGCGTCGT CTTGTCGGTC

```
69421 AGCGCTCCGC CGAAGGCCGT CAGCCAGGCC ACGGCCGGCA CCACCAGGGC CACCACCGCC
69481 GCCACCCGCG CGCGCCACAG CAGGGCCGCG GTCACCAGCA CGGGCACGGC CAGCGCGCTC
69541 CAGGGCAGCA AGGTCTCTGC CAGACTGCTC AGCCGTCCGG GCAGTCCGGG GAGCCGCCCA
69601 TGGCCCGCGA CGCTCACCGC GAGGAAGACG GAACAGCCCG TGACCGCCGC CCCGCGGCGT
69661 CGCCACCACC GCCGGCGCGG ACCGGCGTCC CCCACGCCGA CCGCGGCTCC GGGAGCGCGG
69721 CCCCTGCCGT CCGCCGTGCC GTCCGCAGCC AGGGAGCAGA CGGTCTGTTC CGCCCTGCCG
69781 TCCGCCGCAG TGGAGGGGGC GCTCTCCGCT GCCCCGCTGT CCGGCGCCGC CCGGGAGGGG
69841 ACAGTCTGTG CCGCCCTGCC GTCCGGCGCC GCGAGGAAGA CGGAACAGCC CGTGACCGCC
69901 GCCCCGCGGC GTCGCCACCA CCGCCGGCGC GGACCGGCGT CCCCCACGCC GACCGCGGCT
69961 CCGGGAGCGC GGCCCCTGCC GTCCGCCGTG CCGTCCGCAG CCAGGGAGCA GACGGTCTGT
70021 TCCGCCCTGC CGTCCGCCGC AGTGGAGGGG GCGCTCTCCG CTGCCCCGCT GTCCGGCGCC
70081 GCGGGGGAGG GGACAGTCTG TGCCGCCCTG CCGTCCGGCG CCGCGGGGGA GGGGACGGGC
70141 TCCGCCGTCC CGCCGTCCGG CACCGCGGCC GGATCCCGGC GTGTCGTCGC CGTCATCGTT
70201 CCCCGCCCTG GGTTCCGGCG GCGGCCAGCC GCTCGCGGAC GGCGGTGAGC AGGCCACGGG
70261 CCGCCTCGAC CGCGGCCCGG AGCCCCTCGG CGGGCGTCGT GTTCGCCCGC TGGTGCAGGA
70321 CGGCGCCCAC CAGCAGCCGG GGCGCGCCGC CCACGCGCAC CTCGTAGGCC CACAGGAGGT
70381 TCCCGCCCGC CGGGGTGCTG GAGCCCGTCT TCACACCGAG GACGCCCGGG GTGTCCAGCA
70441 GGGGATTGGT GTTGGTGATC GTCCCGAGGC CCGGGACGGT GGTCTCGCGG GTGGCGACGA
70501 CCGCCCGGAA GACAGGGTCC TCCATCGCGG CCCGCGTCAG CCGCACCTGG TCGGCCGCGG
70561 TGCTTGTGGT CGTCGGCTCG ATGCCGCTCG CCCCCGTGTA GACCGTGTCC TTCATCCCGA
70621 GCCGTACGGC GGCGCGCCGC ATCTTCGTCA CGAAGGCCGC CTGGCTGCCG GAGTCCCAGC
70681 GGGCCAGCAG ACGGGCGACG TTGTTGCCGG AGGGGATGAG GAGCAGTTCC AGGAGCCGGC
70741 GCTGGGAGTG GCGTTCGCCG GACCGTACCG GGACGGTCGA CTCGCCGCCG ACCCCCGCCT
70801 CGTGCGCGGC CGTCCGGTCC ACTTCGATCA GGGGGCCGTC CTCGTCGGGC CTCAGCGGAT
70861 GCTCTTCGAG GATGACGTAG GCGGTCATCA CCTTCGTCAG GCTCGCGATC GGTACGGGCC
70921 GCCGCTCGCC CCGCTCGCCC AGCGAGCCGG TTCCTTCGAG CTCGACGGCG CTCTGGCCGT
70981 CCTGCGGCCA GGGGAGCGGC CCGATGTCGG AGACCGGTAG TCGCTCGCCT CCCGCCGCCG
71041 GAGGCGCGCC GGAGGGCGAG GCGACGGTGA TGCCCAGGGC CGTCAGCAGG GCCACGGACA
71101 GGGCACCGCC GACGAGGCGG TGACGGGGGG TGCGGGGCAG GGACACGGGC CGCCTCCAGG
71161 GGCTGCGGTA CGGGATCGGT ACGGCAGCAA GACTCCGGGG GCTACGTCTC CGCCTCACGG
71221 TCGGGAGAGC GCGGCCCGCG CTCGGGAACC CATCGGTCGT GTATCGGCGG GGCGGCGGCG
71281 ACCGGCCGCC GGGCGACGCG GAGGGAGCGC CTGAGGGGCG GGGCGTACCG ACAGGCGACC
71341 GTCTGGGGTG GGGAGGCCCG CGGGCTGTCC CGGGGACCGG TTCACGCCTC GGACGTCTGC
71401 CCGTCCTCGG GCAGGCTCAG GGTCGCGACC GCTCCTCCGT CCCGGGCGTT GGCGAAGGCC
71461 AGGGTCGCGC CGATCACCCT CGCCTGGCCG GACGCGATCG TCAGGCCAAG GCCGTGCCCG
71521 TGCCCCCGTT CGGCCGAGCC CGTGCGGAAC CGCTGGGGGC CGTGGGACAG CAGGTCGGCG
71581 GGGAAGCCGG GGCCGTGGTC CCGGACGGTC ACGGTCCGGC CGGCGACCGT GACCTCCACC
71641 CGACCCGCGC CGTGCCGGTG GGCGTTGACG ACGAGGTTGG AGACGATGCG GTCCAGGCGC
71701 CGGGGGTCCG ACTCGACCAC TGCCGCCCCC TGTCGCGTCA CCTGCGCCGC GAGGCCCGTC
71761 CGCGCCACCG AGTCCCGGAC GAGGGCGCCC AGGTCGACCG GTCCCTGCTG GGCCGTCTCG
71821 GCGCCCGCGT CGAGCCGGGA GACCTCCAGG AGGTCCTCCA CCAGGTCGCG CAGCACCCGT
71881 ACCCGGCTCT GGACCATGTC CGTCACCTCG CCCTCGGGCA GCAGTTCCGC CGAGGTGACC
71941 AGGCCCATCA GCGGGGTGCG CAGCTCGTGT GCGACGTCGG CGGTGAAGCG CTGCTCGGTG
72001 TCGATCCGCT GCTGGAGGCT GTCGGCCATC GAGTCGACCA CGGCCGAGAT CTCGGCGACC
72061 TCGTCGCCTC CGCGGACCGT TCCCGTCCGG GCGTCGAGGT CGCCCGCCGT GATGCGCCGG
72121 GCCGTGCGGG CGACCCGGCG CAGCCGCCGG GCGGGGAGTT CCGTCGCCAG GGCCGTGGCG
72181 GGGACGACGA CGCCCAGGGT GAGCAGCGAG TACTTCCACA TGTGACGGTC CAGGGCCTGC
72241 CGGGTCAGCA GGTCGGCGGT CATGTCGACC TCGACCGCGT ACAGCTTCCC GCCCTCCCGG
72301 CGGGCCGCCC GGAAGACGGG GGCCGGGGGC CCGTCCTCGT AGAGGGTGGC CTCGCCGCCG
72361 TGCTCGATCT GCCTCAGCAG GGCCTCGGGC AGTTCCTCGG GGGACACCCG GGGCCCTTCC
72421 TCACCTGCTG CGTCGGCGTC CTCCAAGGCC GTTGCCAGCG CCACGTGGGC CCTGCCCGCG
72481 CCCTCGTGCA GGGAGCGCCG CAGCACCGAG TCGTGCACCA GCACTCCGAC GGTCAGCGCC
72541 ATCGAGGCAC AGGCGAGCGC CACCAGGAGG ACGATCTTCC AGCGCAGCGA GCGGGAGCGC
72601 GGTACCAGGC CCGTGACGGC TCCCCGGGGG GCCCGGCTCA CCGCTTCCAC TTGTAGCCGA
72661 AGCCCCGGAC CGTCTCGACG CGCTCGGCGC CGATCTTCTT GCGCAGCCGC TGCACGCACA
72721 GGTCGACGAC CCGGGTGTCC CCGTCCCAGC CGTAGTCCCA CACCTCGCGC AGGAGGGTCT
72781 GACGGTCCAG CACGATCCCG GGGTGCGCGG CGAACTGGAG CAGCAGCCGC AGCTCGGTCG
```

72841 GGGCGAGCGC GATCCGCTCG CCGCCCCGGC GGACCTCCAG GGCCGCGGGG TCGAGGGAGA
72901 GGTCGCCGAA GAGCAGCGGG CCGGCCGGCG TCGCGGGGTC GGCGGGTCCG GGGGCGGGGG
72961 ACACGAAGGC GGCCCGGCGC AGCAGCGAAC GGATGCGCGC CACCAGGACG GCGGTGTCGA
73021 CGGGCTTCAC CACGTAGTCG TCGGCCCCGG CCTCCAGGCC GGACACCACG TCGAGGGCGT
73081 CACCGCGCGC CGACATCATC AGGATCGGGT CCGTGGCCGT CTCCCGGATG CGCCGGCACA
73141 GTCCGATGCC GTCCAGGCCC GGCAGCATCA CGTCGAGCAG CACCAGGTCG TGCCGTCCCT
73201 CCCGGAAGAG TTCGAGCCCG GTCAGTCCGT CGGCCGCGAC GCGCACGCCC TAGCCGTAGC
73261 GCTCCAGGGA CATCGCGACG GACCGCCGGA TCACCTCGTC GTCCTCGACC AGCAGGACGG
73321 TCACGGTCGC AGGCGCGGGC GCCGACACGG AATCAGACAT GTCCATCTCT CGGGCACGGG
73381 GCGGGGCGGG CCGACGCGCG GCCCGGCCCT CCATCATGCC TCACTCGCGC GCCTCCCCCG
73441 GCGCCGCCGG AGAGCCGTCG GCACGCCTCT GAGCTGGTCT GATACCTGAC TGATACATCA
73501 TGGCGCGGTG ACCGACACAC GCCCGAGCGG GGACGGACAC GGAGGCGGCG CGCCGGACAC
73561 AAGATGCCGC GGGAGCCGAT CCGGCGCCCA CCGAGGCCGT GTGAACGACG CCGTCCGGAA
73621 CCGCACGCCC GCGGGGGCTC GTGGCGGGTG TCAGTGGTGT GCGGCAGGCG CGGGCAGCGT
73681 CTCCGGAGCG GGGTCGGTCC TGCGGCCGAG GTGGTTGAAG GCGAGGTTGA GGAGGACGGC
73741 GACCACACAT CCGGTGCTGA TGCCTGAGTC GAGGACGATG CGGGCGCCTT CGGGGAAGGC
73801 GTGGTAGAAG TCCGGGGCGG CGATGGGGAT GATGCCGGCG CCCAGGGAGA TGGCGACGAT
73861 GAGGACGTTG TCGCCGCGTT CGAGGGCGGC TCCGGCGAGG GTCTGGATGC CGCTGGCGGC
73921 GACGGTGCCG AAGAGGGCGA TGCCCACTCC GCCGAGGACG GGCTGGGGGA TGAGCGCGAC
73981 GACGGAGGCG AGCAGCGGGC AGAGGCCGAG CAGGAGGAGG ATGCCGCCGG CCGCGGCTAC
74041 GACGAAGCGG CTGCGGACCT TGGTGATCGC GACGAGGCCG ACGTTCTGGG CGAAGGCGCT
74101 GGCGGCGAAG CCGTTGAAGA GCGGGCTGAG GGCGGTGCCG AGGCCGTCGG CGCGGAGCGC
74161 GGCGGCCAGG GTCTTCTCGT CGGCCGGCCG CTCGACGATC TCACCGAGGG CGAGGACGTC
74221 GGCGGTGGAC TCGGTCATCG ACACGAGCAT CACGATGCAC ATGGAGATGA TCGCCGCGGC
74281 CGCGAACTGC GGAGCGCCGA AGTGGAACGG GGTGGGGAGT CCGATGACGT CGGCGTCGCC
74341 TACGGCGCTG AAGTCGGCGA CGCCCAGCGG CAGCGAGAGG AGCGTGCCGG CGACGAGGCC
74401 CAGCAGGATG GAGATCTGCT TGAGGAAGCC CGTCAGGACG CGGCGCAGGA CCACGGTGAT
74461 CAGCAGGGTG GCGGTGGCGA GGCCGATGTA CGTGAGGCTG CCGTAGTCCG GTGCCTGCGC
74521 GTTGCCGCCC TGGGCCCAGT TGAACGCGAC GGGCAGCAGG GAGACACCGA TGAGGGTGAT
74581 GACCGTGCCC GTGACCACGG GCGGGAAGAA GCGGATCAGT TTGCAGAAGA AGGGGGCGAG
74641 GAGGAATCCG AAGACGCCGG CGACGATCAC GGCGCCGTAG ATGACGGGCA GCGCGTCGTC
74701 GGGGCCTTCG GCCTTCGCTA TCGCGAGCAT CGGTGCGACG CCGGCGAAGG AGACGCCGTT
74761 GACGAACGGG AGCCGTGCTC CGACCTTCCA GAAGCCGAGC GTCTGCAGGA GCGTGGCGAT
74821 GCCCGAGGTG AACAGGCTGG CGCTCATGAG GAACGCGATG TCCGCGGTGG ACAGGCCGAC
74881 GCCGATGCCG ACGACCAGCG GCGGCGCGAC GACGCCGGCG TACATGGCGG CGACGTGCTG
74941 CAGACCGGCG CTGAAGAGCT TCAGCGGGGG CAGCATCTGG TCGACCGGAT GGGTGTCGCC
75001 CGGTCTTGCG GGGGTGTCGG GACGAGCGTC GGTTCCGTCC TCGGTCTTCG TCGGTTCCGG
75061 GGCGGGGCGG GATTCCGGCA TGGCGTGTCT CCTGGCCGCG GCGGTCTCGT GGGAGCGCGG
75121 GCAGCCCTGT GGGAGAGCCG GCGTCGCTGA CGCACGGCTC TGCGGATGGG GGTGGTGACG
75181 GGGTGGGGAG GATGCGGCAC CGGGCGTGGG ACGAGAGGCC GTCGTGCCTG GGTGCCGCGG
75241 CCGGAGAGCG GGGAGTGGGG CGCCGGAGAA CGGGCGGGAG GGATTCGTGT CCCGGGGTTC
75301 CCGCGGTCCT CCGGCCGGCC GGCGCGCAGC TGTCCGTGAT GCGCGTCGTG CCACTCCGTC
75361 CGGCGAAGTC GGAACAGTTC CTCACGGGGC GGCGTGGGTG TCAAGAGGCG GGACGGGCCG
75421 GGTTGAGGAC CTCCCGGACG TGGTGCCAGA GGTGCAGGTC GATGTGTTCG AGGAAGTCCG
75481 CCGCCCGGGC CTCCGCGCGG GCCGCCTGAC GGGGCGGGCC GCCGGCCGCG CCCTCCCATT
75541 GCTGGTAGGT GAGGTGGAGG CGTTCGAGGG CGTGGCCGAG TACGGCGGGG TCGAGCGGCA
75601 CGATGCGGGA AAGGGCCCCC ACGTAGGGGG GCCACCAGGT GGTGGCGGCC TCTCTGAGCA
75661 GGACGCGGAG CCGGCCGGTC CTGCCGCACG CGGCGACGAA GCAGGCCGGT GGCGGGCAGA
75721 GGACTCTCAG GAGGGCGTCC GTCTGGGCGG GGGTCCCCTG CCAGCGGTTC CGGCGTTCGG
75781 CGTCCTCCCA CTGGCCGCGG GTGAGGCCGA GCTCGGTCGC GGTCTCGTTG ACGGTGAGTC
75841 CGGCGACGGT ACGGCACTGG GCGAGGGTGC CGGGCTCTTC GACGAGATCG ACCGGAGAGC
75901 ACCAGAGGGC CGCCGCGAGG GCCTTCACCT GGGTGTTGTC GGGGGTGGCG GTGCCGGCTT
75961 CCCACGCCTC GACGAGGCCG GGGTGCGCGG GGTGTCCGCA GTAGGCGGTG ACGGCCCAGG
76021 CGACCTGGCC GAGCGTCAGG CCGAGCTGGG CGCGGACGGC CGCGGCACGG GTGGGATCGA
76081 AGCGGGGCAG GGCATACGGA TCGTCTGCGG GTGGCATAGC GGGACACCGT AGGGACCCCG
76141 CGGTCCACGG CCAAGGGCCG GAACGGTCTC GATGTCGGCT CCGCCGCCCG GCGTTCGGCT
76201 TCTTCGTCGG ATCTCCTGAG CGGGCAGACC GTCGATGACC TGCTCTTTCA TGGGAGGAGG

76261 CGGGCAAGCG GAGAGAAGAG GAGGCTGAGG CGACTGTCGG TTCCTCCAGA ATCCGCGAGG
76321 AACTGGCGCC TTCTCTTGGG GGAGTTGACG AGGTTCAGGC CGGGCCATAA AGTCCCGTCC
76381 CGTCCACAAC GGAAAACTTC TTCCGTCATG CGAAACCTGT GGTGACAGCC GCCCACCCCC
76441 GCGGCTCGCC CCCATCGGCC ATGCTCCCGG TTCGGGCCGT CCCCCGGCAC CGGTCACTCT
76501 GCCCACACGC CCCCGGCAGT CCGCTGCCCG CGGGCGGTGT CTCAAGGACC TGCCTTGCTG
76561 CCCCGCGCCA CTGACCACGC ATCGCCGACC CCACCCCATC CGGTCGACGA GATCCTTCCC
76621 GCCCGCCGCA TGCTGCCCGC CGCCCTCCAA CACGTGGCGA GCATGTACCC CGGCCTGACC
76681 GCACCACCGC TGATCATCAG CAGCGCCCTG GGGCTGACCC CGGCCCAGCT CTCCGCCCTC
76741 CTGGCCGCCG CGCTGCTGAT CGCCGGGCTC GGCACGATCG CCCAGACCCT CGGCGTCTAC
76801 GGCGTCGGCG CCGGGTTGCC CCTCGTCAAC GGCGTCTCGT TCGCCGTCGT GTCACCGGCG
76861 CTCGCCACCG CCGCCACCCA GGGGCGCGAC GGCGCCCTCC CGGCGATCTT CGGGGCCACC
76921 CTCGTGGCGG GACTCCTCTG CCTGCTCCTC GCTCCCGTCT TCTGCCGACT GGTCAGGTTC
76981 TTCCCACCGG TCGTCAGCGG CTGCGTCATC ACCCTGGTCG GCATCTCCCT CCTGCCGGTC
77041 GCCGGCACCT GGGCCCGGGG CGGAGACGCC GAAGCCGCCG GCTTCGGCTC CCCCGCCGAC
77101 CTGGCCCTGG CGGCGACGAC CCTCGTCATC ACCCTGACCG TGCACCGCAT GCTCTCGGGC
77161 CGCTTCCTCG GGCGGGTCGC CATCCTCATC GGCATGCTCG CGGGCACCCT GATCGCGATC
77221 CCGCTGGGCA AGGTCGACCT CGACCCCCTC GCCCAGGCGC CCCTCTTCGC CCTGCCCACG
77281 CCCTTCGGCT TCGGCACCCC GCAGTTCGTC CCCACCGTGA TCGCCACCGC CGCGGTCGTG
77341 ATGATCGTGT CCATGATGGA GTCCACCGCC GCGCTGCTGG CGCTGGGCGC GGTCGCCGAA
77401 CGGCCGGTCC GGGACCGGAC CATCGCCGGA AGCCTCCGCG CCCTCGGCCT CGCCACGGTC
77461 CTCGGCGGCG TCCTCGGCTC GTTCACCAGC ACGTCGTACG CGCAGAACGT CGGCCTGGTC
77521 TCCCTCAGCC GGATCCGCAG CCGCTATGTG GTCACGCTCT GCGGCGCCGT CCTCGTCCTG
77581 ATGGGCTTCG TGCCCGTCCT GGGCTCGTTC GTCGCCCTCG TCCCTCTGCC CGTCCTCGGC
77641 GGTGCGGGGG TGGTCTTCTT CGGCTCCGTC GCCGTCACGG GCATCCGTAC GCTGGCCAAG
77701 GCCGCCCTCG GCACCGGACA CAACGCTGTG ATCGTCTCCG TCACCCTCGC CTTCGGTCTC
77761 TTCCCCGTCC TGGACCCGGA CTTCTACGCC CGTCTTCCCG CCCCGGTGGC GACCGTGCTC
77821 GGCTCGGGGA TCACCGCCGG CTGCCTGGTC GCGGTCCTCC TCAACTACCT CCTGAACCAC
77881 CTGGGCCGCG GCACCGAGGC CGACCCCGAC GCGATCTCCG CGGAACAGGT CACCGCCCTC
77941 GACACCGCGG ACACCGTCCT CGGGCCGAAG CGTTCCTCCG ACTGGACGCC CTTCCAGCCC
78001 TCCGGCAGCC CCTCCGGCAC CCCTGACCAC GGCCGTCACA CCAGGGGCAC GGCACGGCCC
78061 GCTCCCGCCT GGCCCTACGT GACCGGCCCC GTGGACCCCA CCGACACCGG GCGGCACCAC
78121 CGGCCGCACG AGGTTCCGGC GCCGCCCCAC CGACCAGACG AGGTGCCCCC GCCGCTCCAC
78181 CCCTCTGCCG CTCACGAAGG CGAACCCCCG CCCGCCGTCA CCGAGAACGC GGTCTTTCCC
78241 GGACCGCTCC ACCCGCTCCA CCCGCTCCAC CCCCGGCCCA CCGGTCGTCC CGACCGTCCC
78301 CGGCAACGGC ACAGTGCGGA GGCCGACCCC TGGCAGCATC CGCAGACCCC CTCCGCATCC
78361 GGCGACAGCC AGTAGAGACG ACCTCCCCCG ACCTCTTCGC AGAGCCCGGC ATCGCACAGG
78421 CCCGGCGGAG AGGTGGCGCC GACCCACCCG ACCACCGCCG GAAGCGCCCC CGGGGACCCG
78481 TGTCCCACGG ATTCCCCGGC GACAAGACGA GGTAGCCCCG ATGACCACCG TTTCCGCCGC
78541 CCGCCACCGT GCGGGCGGCT CCCCGCGCGG CGGCACGTCC CGCCCGGGCC CCGACGAGAG
78601 AATCGCCCAG GTCGTGGCCG AGGCCCTCGG ATCCGCCCGG ACGGTCCTCG ACCCGGATGC
78661 GCTGCCCGGC CTCGGCACCA CGCGACTCCC GTTCGGCGAC GGGAGGTTCG ACGCGGCGAT
78721 GATGCTCTGC AACGCCCCCG GCGTCCCCGA CGCGCTCTCG CGGCTCGGGG AACTGCGCCG
78781 CGTGACACGG GGCCCCGTCG TGGTCCTCGC GACCGACCCC TCGCGCGTCC GCTCGTTCTG
78841 GCTGGACCGG TACGCCCCCG AGGTCCTGGC CGTCGAAGCG CGGCGTCATC CGCCGATCGC
78901 CGATCTGACC GCCGTCCTCG GGGGTTCCGC CGAGGTGCGG AGCGTCCCCG TTCCCCTCGA
78961 CTGCACCGAC ACCTTCGACG AGGCGTACTA CGGAAGACCC GAGAAGCTCC TGGACCCGTC
79021 GGCCCGCCAG GCGGGGTCGG CCTGGAGCTT CGTGGACGAC CGGGTCCGCG AGGAGTTCGA
79081 CACGACCCTG CGCCGCGAAC TCCGGTCGGG GGAGTGGGAC GAGCGCTTCG GCCACCTCCG
79141 CCGCCGGCCC GTCTACGAGG GATCACTGGT GATCGTCCGT GCCGTCCCCT GACGTCCTCC
79201 CGGGGACGCG CCACCCCCGG GGTCGCGTCA GCGGTCTCCG GCCAGGTGCC CCGAGAGCTC
79261 GCGCATCAGC GTCTCGTGCT GCTGGAGCAG GTAGAAGTGG CCGCCGGGCA GGACCCGTAC
79321 CCGGAAGCCC TCGGGTGCGA CATCCGCCCA GGCGTCCATG TCCCCTACCG CCACGTTCGG
79381 ATCCGTGTCG CCGATCCAGG CGTGCACCGG ACAGCCGACG GCGGTGGGGA CGCGGGGGCC
79441 GTAGGTGCTC ACCACGGTGA AGTCCGCCCG GACCGCGGGC AGCACGAGCT GTCGGATGTC
79501 CGGGTCGTCA AGCAGGGCGG TATCGGTCCC GCCGAGCCCG CGCAGTACGG CGACCAGCTC
79561 GTCGTCCCCC TTCCGGTGCA GGTCGAGCGG GGTCAGCCGG TGCGGGGCCT TGCGGCTGGA
79621 GACGTGGAGG GCGGCGGGCG TGACGCGGTG CCGCTCCTCC AGGCGCAGCG CCACCTCGTA

79681 GGCCAGGGAC GCGCCCATGC TGTGGCCGAA GAGCGTCAGG GGCACGTCCG CGAGCGGCAG
79741 CAGTGCCGCC GTGACCCGGT CGGCGAGCAC GTCCATCCGG TCGACGAACG GCTCGTTGAA
79801 CCGCTCCTGG CGTCCGGGGT ACTGCGCCAC CAGGACCTCG GTGTCGCCGC CGAAGGCGCT
79861 GCCCCAGGCG TGGAAGAAGC TCGCGGAACC GCCCGCGTGC GGCAGGACCG CCAGCCGCCG
79921 CCGGGGCGCG GGAGTGCTGG AGTACCTGCG GAACCACGTC GTGCTGTCCG TGCCGGTCGT
79981 CATGTGTGCG TACACCCCGT CCTCGGGTTC TTGGGGTGCC AGTGTCCCCG CAGGGCCCGG
80041 TGTCCGGACG CGGTGGGGGT CCGGTGGCGA GCCGCTTACG TGTCCCCGCG CTTCCGGGAC
80101 CGGCGGCCGC ACACGTGTCG GCCCCCACGA ACACCAGGGT GCGTGGGGGC CGATGCGTGT
80161 TTCGAGTCCT GGTCTGACGA TTTCAGGCCG AAAGATATGT CGGACTTTAC AGCTGCGATC
80221 GAAGCCGATC GATAATGCCG TGGACGGGTA ACGTCGGAAT CACTCGGTGC TCTTGAGCGC
80281 ACCACTCACG TTGACGACCT CGTGGCACTC CCGCGCCTGC TGTCCCGTCG CGGGCGTACC
80341 GGGCTTCGTG CAGGTCACGT CGATGGTCAC GGTGTCCTTC GCGCCGATCC TGATGGGAGT
80401 CACCCAGTGG TAGTCCTGGT TGCGGAACGT CTCCAGCGCG ATCGTGGTGA TCTTGCGGTC
80461 CCCGAAGGTG ATCGTCATCA CCCCTTCGTC ACCCTGGAAG TTCGCGACAA CGATGTCCGT
80521 GATCCCGAAC ACGGTCTTCT CGGGGACCGT GTACGTCCCG GTCCTGGACT GTCCCGCTCC
80581 CGACCTCAGG TCGATGGTGG CCGAGCTCTG CCGTCCCCCG CCGGTCGCGG TGCCGTCGCC
80641 CGTGCCGGCC GAACCGTCGT CCCCGCCGCC CGCTCCACCG CCCGATCCCG GCTTCTGGCT
80701 GCTGCCGCCG GACGGCCGGC CCGGGCCGGG GGAGGAGCCG TCCGAGCCGC TCTCCGTCGG
80761 GACCGGGCGG GGCTGCACCG CCTGGGTGGC CGCCTCCTCG GCGGCGCTGC GCACGGCCGG
80821 CCGGACCAGC GTGAACCAGG CGATCAGCAG CGCGATCAGC GCCGCGAGCA GCAGGAGCAG
80881 CCACTTGGGG AAGACCGGTA TCTGGACGAA CTCCGCGTCC AGCGTCGGCG CCGTGTGGGG
80941 CTCCTCGGCG CGGTCCTCGG TCTGCTCCGG CTCGCCGGTC TCGCGGGCGT CCACGGTGAA
81001 CGGCCAGACC ACGGGGTCGC CGAACCACAC CGGGCTCGCG GTGCGGACCC GCAGCCGGAG
81061 TTCCTTCGAC TCGCCCGGCT CCAGCGCCGG CTCCGCCGGC GTGAAGGCGA ACCGGAGCTC
81121 CTCGCCCGCC TGCCCGGGCG TGAACCCCAC CCGTACCGGG GTGTTGCCCT GGTTGCGGAC
81181 GGCCAGCAGA TAGCGGCCCC GGAGCCAGCC GCGCCGGCGG CGCGGCGAGA GGTCGGTCCG
81241 CAGCTCGTGG AACGCGCCGA CGCGCACCAC GGTCTCCAGG ACCTTGACCG ACTCGGGCTG
81301 CTCGTTCGGG AGGATCCGTA CACCGAGGGG CAGCTCGCCG GCCCGTGTCT CCGGCGAGCG
81361 CGGCGGTGCC AGACGGAGCG TCACCGTCTC GGACGTGCCG GGATAGAGGG AGAGCCGCTC
81421 GGGCTCGACG GTGGTCCATT CGGCACCGTC ACCGACGACC TTCAGGTCGT ACGCCTCGAC
81481 GATGTCACTG TCGTTGCGGA CGGTCAGGGT GGTGGTGGCG ATGTCGCCCG GCGTCACGGA
81541 CACGGCCGGG ATGTCGAGGC CGGGCGCACC GGGACCGGAG GAGGCTGCGG AAGGCGTCAC
81601 CCGCCCCACC GTAGGAGACC TGACAGATCC GTACGAGGCA CGCGAGGGCA ATGTCCGGGC
81661 AGCTCGGCTG CCCGGCAAGC ACAAGTCAAC TCTCCGGTAA CAATGGATTT CTAGTCTGGA
81721 GAGCCGCCTT CGGCACACCA CCGGCCCGTG GTCGGCTCGT GTCGTGTCCG CCTTCCCCCC
81781 ACCGACCCAG GAAAACAGGT ATCCGATGTT CCGCACCGAG GAGAAGAGGC CGGTCGCGAC
81841 CGGCACTACG GCGCATGACG CCGTCCGGGG CCACCCGGAC GCCCATGCCG CCGGCTTCGG
81901 CCGCCCGCGC CGCGTCACCG TGGCGGTCTA CGCCGCCGAC CCCGTGCTGC GGGTCGGCGT
81961 CGTCCAACAG CTCCGCCAGC GCCCCGAGAC CGAGCTCGTC GACGACGCGG ACGCGGAGAA
82021 CGCGCAGGTC TCCCTGGTCG TCGTCGACGC CCTCGACGAC GACGTGACCG CCCTGCTGAC
82081 CCGGCTGAGC TACAACGGCG CCACCCGCGC GGGACTCGTG ATCGGCACCC TCGGCGTCGG
82141 GGCGCTCCAA CGCGTCGTCG AGTGCGGGGT GTCGGCGGTG CTGCGCCGCG CCGAGGCCGA
82201 CCAGGACCAG CTCGTCCAGC TGGTCCTGGC GGTGGCCAAC GGCGAGGGCG TGCTCCCGGG
82261 CGACCTGCTC GGCGAGTTAC TGGGACACGT CGGCAGCCTG CGCCGCGCGG CCCTCGACCC
82321 CGGCGCCCTG CCCCTCTCCA CCCTCACCAG CAGGGAGGCG GAGATGCTGC GCCTGGTCTC
82381 GGAGGGCCTG GACACCGCGG CGATCGCCCG CAAGACCTCG TACTCCGAGC GGACCGTGAA
82441 GAACGTCCTG CACGAGATCA CCACCCGCCT CCAACTGCGC AACCGCGCCC ACGCCGTGGG
82501 CTACGCGCTC CGCAACGGGC TGATATGACC GTCCCGTCCG GACCGCGGCC CGGCGGCCGG
82561 CGCGACAGCC GGAGGGAAGG CGGCGCTGCC CCAAAGTGCA TCCCGCCCTT CCCCCGGGTG
82621 CGGCCCCCGG GCCCTCCCGC CGCGTGCGCC GCCGCCGCAC GATGACGGGG GGCACCTCCC
82681 GGTGCCGCAC CGGACGGAGA AGGGCACCGT GATGAAGACC GCTGGCCCCG GTGGACGGCA
82741 CCGCCGGGGG AGACTCGCCT CGGCGCTCCT GCTGCTCGTC CCCCTGCTGG GCGCGACGGG
82801 CGTGGCCGGG CCGGACGACC CCCGGACCGC GGCGGCCGCG GCGGACGCCG CCGAGACCAC
82861 CCGCATCGCC TACGCGGGCA CCGGCCACCG CAGCCTCGGC GAACCGGCCT CCACCGACTC
82921 CAGCACCCCG CTGTTCGGAG CGGGACCCAC CCACTACGAC ACCGACCCGT CCGCCCTCGG
82981 CGACCGGCTG GTCTTCGCGA GCCGCCGCGA CGAGAAGCAC CCCCAGATCT ATCTGCGGGG
83041 CGCCGACGGC GGAGTCCTGC GGCTCACCAG CGGCCTGGAC GCGGCCCGTC CCCGGCTCAC

```
83101 CCCGGACGGC GGGTCGGTGC TCTTCGACGC CGCCGACCCG GCCGGCGGCT CCCAGCGCGA
83161 CCTGTGGCTG GTGCGCACCG ACGGCACCGG GCTGACCCGG CTGACGGACA CGCCCGCCAG
83221 CGAGGAGGAC CCGGCGGTCT CCCCCGACGG CGCCCGGATC GCCTACTCCA GCGACGCCGA
83281 CCCCCTGGCC GGGCGGCAGA TCTACGTCCG CGCCCTCACG GGCGGCATCC CCACCCGGCT
83341 CACCGACCCG GCCCGCGGCA CGGCCTCCGA GCCCGCCTGG AACCCCGTCG ACGACGACGT
83401 CAACCGCGCG TGGATCGCGT ACACGTCGAC CACGACCGAG GACGGGCGGA CCAGGCAGCG
83461 GCTGCGGATC ACCGACGGCA CCACCGACGA GACCCTGTTC ACCGGCGCCT ACGCGAACTG
83521 GCAGGGCCAC GGGGCGGCAT GGCTGCCCGA CGGGGACGGG ATCGTGTTCC TCAGCCCCGA
83581 GACCACCTGC ACCTGCAGGA CCCCCTACGA CCACGTCTTC CGGTCGGTCG TGCACGCCGA
83641 CCGGGAACCC TCCCTGGTGC TCGACGAGGA CCGCGACGTC CTCTCGCCCA CCTGGATCGG
83701 CACCGCCGAG GGCGGCCACG CGATCGTCGA GCGCAGCTCG GCGGCGACCG CGCACACGGC
83761 GACCCTCCAG GACATCCGCG CGGACGGTTC CGACCCGCGC GACCTGCAGC GGAAGATCCT
83821 GCGCGAGGAC CCCCAGGCCG ACACCAACAC CGACCCCGCC AAGGATCCGC TCTTCCAGCC
83881 CGCGCCCCCG TTCGACCCGT GGACCGAACG GCAGAACTAC ACCCCCGACG GGCGCCGCCT
83941 CGTCCTCACC CGCTTCGAGG GCCCCGACGA CGCGCGGATC GAGCGGATCT GGACGGCCGA
84001 CGCCGACGGT ACGAACGAGG CGCCGATGCC CCTCGACGGG CGCGGCGCGC GGGACTGGGA
84061 CACCGACCCG ACGTTCTCCC CGGACGGCAC CCGCCTGGCC TTCACCCGCA CCTCGCCCGG
84121 CGGGGTCGGC GAGGCCGCGG GAGACAGCCG CATCCTCCTC GCCGAGGTCG CCACCGGCCG
84181 GATCACCGGA GAGATCGTGC CCCCGGCCGG TGAACTCCGC GGCGGGGACG CCCAGCCGAC
84241 CTGGTCCTCC GACGGCACCA CCCTGGCCTT CACCCGCGCC CGGCAGATCG CCGGGGGCGG
84301 CGGCAGCAAG CACGTGTGGA CCGCGTCCAC GGCTGACCTG ACCCGGCAGC GCGACCTGAG
84361 CGCGACGCAC TGCCCGCGCG ACTGCGACGT CATCGACGAC AGCCCCGCCT TCTCGCCCGA
84421 CGGACGCTCC CTCGCCTTCA ACCGCAAGAA CGGCGGCGGG CGGATCGACG AGCGCAACGG
84481 ACTGCTCCTG ACCACCCTGT CCGGCGACGC CTGCCAGGTC CTGCTGCCCA CCGCCGCCCG
84541 CGGCCAGGAC GGCGCGTGCG AGCGGGAACT GCCGGACACC ACGCTCACCG GTCCGCACCA
84601 GCCGCGCGAC GCCGCCTGGA CCGCCGACGG CAAGAGGCTG GTCTTCAGCT CCCGGGCCGC
84661 GGCCGCGGTC AACAGCCCGG AGAAGCTGAA CGTCCTGGAC GTCGGCTCCG GTGACATCAC
84721 CCCGCTCACC GCCGAGCTCG CCGGACGCCA GAAGGAACCC ACCGTCCAGC AGTCCGTGGA
84781 CCTCGCCGTC GAGGCACCCG CCACGACGCC CGACGTCACC GTCGGCGCGT CCGGCACGGT
84841 CACCGTCCAC GTGGTCAACC ACGGTCCCGC CGCCTCGCCC GGCACCCGGC TCACCGTCGT
84901 CCCGCCGTCC GGTGTGCGGA TCACCGGGAT CGAGTGGCCC GGCGGCACCT GCGACGCCGC
84961 CTCCCTCCAG TGCGACCTGG GCGTCGTCGA GGCCGGAGCC CAGGTCCCCG TGGACGTCAC
85021 GCTCACCGGC GTCACCGCCG GCGACGCACC CGTCGACTGG TCGGTCACCG GCGCCGTCCT
85081 CGACCCCCGG CCCGGCGACA ACGACGGCCG GAGCGTGATC CCCGTACGCG AGGCACCCCC
85141 GACGCCGACC CCCACGCCGA CGCCGACCCC CACGCCCACC CCGACCCCGA CTCCGACGCC
85201 GACCCCCACC CGGACCCCGA CGCCCACCCC GACTCCGACC CGGCCCCCGC AGCCCCCCGC
85261 GCCGAAGGCC GGACCCGGGG TGCGGATCAC CGTCCAGCCC GAGCCCGGCT ACGTCGGCGG
85321 ACGCGTCGTC GTCACGTACA GCGTCCGCAA CGGCCGCAAC GCGCTCGCCA CCGGACTCCG
85381 GCTCAGGATC GGACTGCCCG CCGGGGTGCC CCACGGCGGA CTTCCGGCGG GCTGCGACCG
85441 GAACGGCGCG TGCGCGCTGC CCGACCTCAC CCCGGGCACG ACCGCCGTCC TGCGGGTCGT
85501 CCTCAGCCCG AAGAAGGCGA TGACCGCCCG CGTCACGGCC GTGCTCGACA CCACCGGCAC
85561 GGACGCCGAC CGCAGCGACA ACACCGCCCG GGAGCGGCTG CGCGTCCTCC AGCCGCGCAT
85621 CGTCGCCGTG CCCGACATCG GCAAGCCCGG ATTCGTCACC TCCGTCCGAG GCGTGGACTT
85681 CCCGCCCGGC GTCCCGGTGC GCTTCAGCTG GAACCCCGGG ATCACCGCCG CCGCCTCGCC
85741 GACCTTCCCG GAGGCCGACG GCACGTTCAT CGGACAGCTC CTCATCCTCG CCAAGGACCA
85801 GACCGGGCCG CGCACCATCA CGGCCTCGGG CCCCGGATTC TCCCCGGTGA AGACCGACTT
85861 CCTGGTCGTC AGCGGCACCG TCCAGCCGCC GGACGGGGTG ACTCGCCGGT GATCC
```

Example 2 Construction of a "Clean" Host Strain, *S. fradiae* K159-1

**[0102]** This example describes the preparation of the clean host, *Streptomyces fradiae* K159-1, a strain in which the *tylGI, tylGII, tylGIII, tylGIV*, and *tylGV* genes have been deleted. Plasmid pKOS159-5 was first constructed as follows. Two fragments flanking the *tylG* genes were PCR amplified from *S. fradiae* genomic DNA using the following primers:

*tylGI* left flank:
forward 5'-TTTGCATGCGATGTTGACGATCTCCTCGTC [SEQ ID NO:__];
reverse 5'-GGAAGCTTCATATGTTCTCTCCGGAATGTG [SEQ ID NO:__];

*tylGVI* right flank:
forward 5'-TTAAGCTTTCTAGAGAGGAGAGGCCGTGAAC [SEQ ID NO:__];
reverse 5'- AAAGAATTCGAACTCGAGCACGGACTCGTTG [SEQ ID NO:__].

**[0103]** The *Sph* I, *Hin*d III and *Eco*R I restriction sites are underlined. The two fragments were then cloned into pSET152 using the underlined restriction sites and corresponding sites in pSET152 [see Bierman, M., Logan, R., O'Brien, K., Seno, E.T., Nagaraja, R. & Schoner, B.E. (1992). Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* 116:43-49]. The resulting plasmid was named pKOS159-5. This plasmid no longer contains the *int*-ϕC31 gene and *attP* locus from pSET152 and therefore serves as a suicide vector for delivery by homologous recombination.

**[0104]** Spores of *S. fradiae* 99 (Russia) were prepared by harvesting from strain grown on 1-2 AS-1 plates [see Wilson, V.T.W. and Cundliffe, E. (1998). Characterization and targeted disruption of a glycosyltransferase gene in the tylosin producer, *Streptomyces fradiea*. *Gene* 214: 95-100], filtering the spores through sterile cotton, and resuspending in 1 ml of 20% glycerol [see Hopwood, D.A., et al. (1985). *Genetic Manipulation of Streptomyces: A Laboratory Manual.* The John Innes Foundation, Norwich, UK]. Spore suspensions were stored at –20 °C. A 20 µl aliquot of the spore suspension was added to 5 mL of 2xYT and incubated at 30 °C with shaking. After two days, the cultures were diluted 1:50 and incubated at 30 °C with shaking for 3 h. After that 1 mL of the cultures were collected by centrifugation (recipient cells). Donor cells were prepared by transforming *E. coli* S17-1 with pKOS159-5 and selecting for apramycin

resistance only. Several colonies were picked off the primary transformation plate and used to inoculate 5 ml of LB with chloramphenicol (10 μg/ml) kanamycin (100 μg/ml) and apramycin (60 μg/ml). The cells were grown at 37 °C for 4 h ($OD_{600}$ of 0.4-0.6), collected by centrifugation, washed in 5 mL LB, centrifuged, and resuspended in 100 μl of LB. Conjugal transfer between the donor and recipient cells was performed by resuspending the recipient cells in the 100 μl donor suspension and spreading the cells on AS-1 plates. After incubated at 37 °C for 16-20 h the plates were then overlayed with 3 mL of soft nutrient agar containing 1 mg nalidixic acid and 1.5 mg apramycin. Exconjugants were observed after 48 h of further incubation at 30 °C.

**[0105]** Apramycin resistant colonies were analyzed by PCR to confirm single crossovers at both flanking regions. One colony was selected for carrying out a double crossover as follows. The strain was grown on AS-1 plates non-selectively until well-sporulated. Spores were harvested, dilutions were plated on AS-1 plates, and single colonies were screened for loss of apramycin resistance. A single apramycin sensitive colony was isolated which did not produce tylosin. The double crossover was confirmed by PCR. This strain was named *S. fradiae* K159-1.

**[0106]** *Streptomyces fradiae* K159-1 was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 12 March 2003, with accession number PTA-5060.

Example 3 Construction of *S. fradiae* K159-1/244-17a, A "Clean" Host Expressing Methoxymalonyl Biosynthetic Enzymes

**[0107]** *Streptomyces fradiae* K159-1/244-17a is derived from strain K159-1 (Example 2) by addition of the *fkbGHIJK* genes from *Streptomyces hygroscopicus* var. *ascomyceticus* ATCC 14891, which encode proteins catalyzing the biosynthesis of methoxymalonyl-ACP.

**[0108]** The putative methoxymalonyl-ACP biosynthetic genes from *S. hygroscopicus* ATCC 14891 (*fkbGHIJK*) are arranged with the 3' end of *fkbG* (encoding an O-methyl transferase) overlapping by 6 codons the 3' end of *fkbH* (encoding an unknown function), which is the last gene of a convergent operon that begins with *fkbB* (one of the PKS genes) and ends with the genes *fkbK, J, I* and *H*. To facilitate expression of these genes in *S. fradiae*, an operon was constructed beginning with *fkbK* and ending with *fkbG*, all in the same direction. This was done using PCR to clone *fkbG* with flanking restriction sites to allow its 5' end, with its existing

Shine-Dalgarno sequence, to be fused to the 3' end of *fkbH*. This operon was then placed behind the *tylG* promoter in a pSAM2-based vector, which was introduced in *S. fradiae* clean host K159-1 by conjugation. Exconjugants were selected and named K159-1/244-17a.

**[0109]** *Streptomyces fradiae* K159-1/244-17a was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 12 March 2003, with accession number PTA-5053.

Example 4 Construction of an operon containing all five chalcomycin PKS genes and expression in *S. fradiae*

**[0110]** A construct comprising the genes encoding *chmGI-V* was constructed as follows: The 3' end of ChmGV was obtained by PCR with pKOS146-185.10 as the template and the following primers (Chalco-1A: GACACGGCCGGTGAGAGCAGC [SEQ ID NO:__] and Chalco-1B: CTTCTAGATGTCGCGGTGTACGG [SEQ ID NO:__]). The 942 bp PCR product was digested with NcoI and XbaI, the 309 bp fragment was gel isolated, and the fragment ligated into the same sites of Litmus29 to give pKOS342-33. That plasmid was cut with NcoI and XhoI and ligated to a 2.4 kb NcoI-XhoI fragment from pKOS146-185.10 to give pKOS342-35. That plasmid was digested with BglII and XhoI and ligated with a 6.4 kb BglII-XhoI fragment (including *chmGIV* and the 5' region of *chmGV*) from pKOS146-185.10 to create pKOS342-36 (containing *chmGIV* and *GV*).

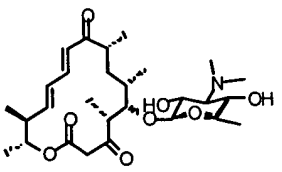
**[0111]** A 5.4 kb HindIII/ PstI fragment containing the 5' half of *chmGIII* was isolated from pKOS146-185.1 and a 6.3 kb PstI/BglII fragment containing the 3' half of *chmGIII* was isolated from pKOS146-185.10. These two pieces were ligated into Litmus28 cut with HindIII and BglII to obtain pKOS342-38. Plasmid pKOS342-36 was cut with BglII and SpeI, the 9 kb fragment was gel isolated and the fragment ligated to the BglII and SpeI sites of pKOS342-38 to obtain pKOS342-39.

**[0112]** Plasmid pKOS232-172 (described in Example 5), containing *chmGI and GII* was cut with NdeI and HindIII and the 19 kb fragment was isolated. Plasmid pKOS342-39 was digested with HindIII and SpeI and the 20 kb fragment was isolated. These two fragments were then ligated into the vector portion of an expression cosmid, pKOS244-20 (gel isolated 8 kb NdeI-SpeI fragment). The resulting plasmid (pKOS342-45) was recovered using in vitro λ phage packaging (Stratagene) and infection of E. coli DH5α. The correct clone was identified by

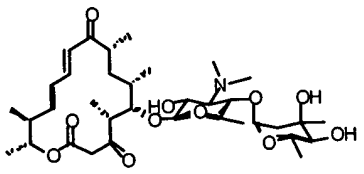
restriction enzyme analysis and the plasmid was moved into *E. coli* DH5α/pUB307 and conjugated into *S. fradiae*.

**[0113]** Expression of PKS genes in *S. fradiae* (in this and the following examples) were under the control of the tylosin PKS promoter (tylGIp, see Rodriguez et al., "Rapid engineering of polyketide overproduction by gene transfer to industrially optimized strains" *J Ind Microbiol Biotechnol*).

**[0114]** Apramycin resistant colonies were obtained, shown to secrete bioactive compounds, and grown vegetatively in 5 mL TSB medium (+ 30 μg/ml apramycin) at 30°C for 48 h. Two mL of seed culture was inoculated into 40 ml Russia (R) medium (15 g/L whole wheat flour, 10 g/L corn gluten hydrolyzate (Sigma), 25 g/L beet molasses, 2.5 g/L brewer's yeast, 1 g/L (NH4)2HPO4, 1 g/L NaCl, 2 g/L CaCO3, and 34 g/L soybean oil) in 250 ml baffled shake flasks. After a 7 days growth at 30°C, the culture broth was analyzed for 16-membered macrolide production by HPLC (Metachem Metasil Basic column, 4.6x150 mm, 5 μm particle) using linear gradient from 15 to 100% organic phase (56% methanol, 5mM ammonium acetate) at 1 ml/min over 7 min. The HPLC used simultaneous detection by electrospray mass spectrometry (Turbo Ionspray source) and UV absorption at 282 nm. LC-MS analysis of the broth showed that several chalconolide derivatives were produced. The most abundant compounds were purified and shown to have the structures below. The 3-keto also forms the enol tautomer.




524 amu




668 amu

Example 5 Construction of *Streptomyces fradiae* K232-192 Expressing a Hybrid Chalcomycin-Spiramycin PKS

**[0115]** *Streptomyces fradiae* K232-192 is derived from strain K159-1/244-17a (Example 3) by addition of hybrid chalcomycin-spiramycin PKS genes, which encode proteins catalyzing the biosynthesis of 14-methylplatenolide. The chalcomycin genes were obtained from cosmid pKOS146-185.1, which was deposited under the terms of the Budapest Treaty with the American

Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 19 February 2003, with accession number PTA-4961.

**[0116]** The first two genes of the chalcomycin PKS were isolated from the cosmid pKOS146-185.1 as EcoRI/XhoI and XhoI/BspHI fragments, and a coding sequence for a spiramycin PKS C-terminal linker attached to 3' end. The EcoRI site is near the 5' end of chmG1. The EcoRI/XhoI fragment was cloned into a modified Litmus28 with a synthetic linker inserted in order to create an appropriate translation start sequence. The altered region of the Litmus28 polylinker between the AflII and EcoRI sites in this plasmid (pKOS232-165) is given below. The plasmid with the chmG fragment was pKOS232-168A.

```
AflII      PacI    SD       NdeI        EcoRI
CTTAAGGGTTAATTAAGGAGGACACATATGTCCGGAGAATTC [SEQ ID NO. __]
                             M  S  G  E  F
```

**[0117]** The XhoI/BspHI fragment was ligated between the XhoI and NcoI sites of Litmus28 to give pKOS232-156. The two fragments were then joined to give pKOS232-172.

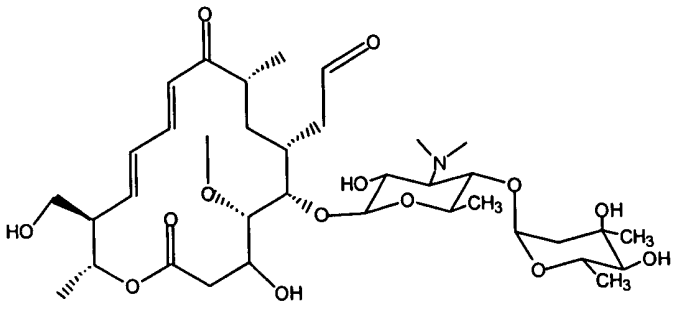
**[0118]** Starting with overlapping cosmid pKC1306 (described in US Pat No. 5945320 to Eli Lilly Company), a cassette containing the last three ORFs of the spiramycin PKS was constructed as follows. An AvrII site was introduced at the 3' end of *srmG5* by PCR from a natural MluI site to the 3' end. The PCR product was cut with MluI and AvrII, gel isolated and ligated into a Litmus-based vector (pKOS232-75B) between the same sites to give pKOS231-118A. The 7 kb BamHI/MluI fragment from cosmid pKC1306 was subcloned in Litmus38 (New England Biolabs) to give pKOS231-113A. The 3.8 kb BamHI/MluI fragment of pKOS231-118A was gel isolated and ligated with the 7 kb BamHI/MluI fragment of pKOS231-113A to give pKOS231-120. The 7 kb BsrGI/BamHI fragment from pKC1306 was subcloned in Litmus38 to give pKOS231-113B. The 6.2 kb PstI/BamHI fragment from pKOS231-113B was cloned into Litmus28 to give pKOS231-122. The 7.5 kb BamHI/AvrII fragment was isolated from pKOS231-120 and ligated with pKOS231-122, which was cut with BamHI and AvrII and dephosphorylated, to give pKOS231-124. The 3.1 kb BamHI/SpeI fragment from pKOS231-118B (which contained a PCR fragment that created a 5' end for *srmG3*) and the 7.5 kb BamHI/AvrII fragment from pKOS231-120 were isolated and ligated to give pKOS231-130. The 14 kb BamHI fragment was isolated from pKC1306 and subcloned in Litmus28 to give

pKOS231-111B. The 14 kb BamHI fragment was isolated from pKOS231-111B and ligated to pKOS231-130 cut with BamHI and dephosphorylated, to give pKOS231-132.

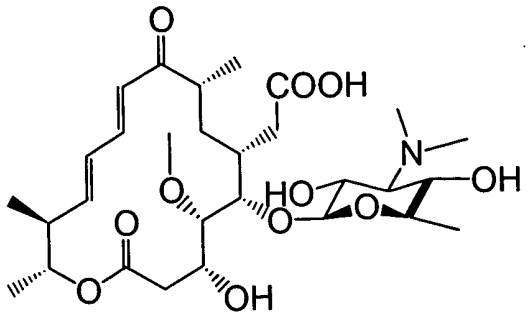
**[0119]** To attach a coding sequence for a spiramycin PKS C-terminal linker to the 3' end of *chmGII*, a HindIII site was introduced at the 3' end of *srmG2* using PCR with pKOS231-112B as template. The engineered HindIII site was positioned with respect to the reading frame to match that of the natural HindIII site in the chalcomycin *chmGII* gene. The resulting PCR product was cut with HindIII and BamHI (a natural site) and ligated into the same sites of pKOS231-114A to give pKOS232-178. This was then joined to pKOS231-132 at the BsrGI site to give pKOS232-182. The chmG1,2 cassette was isolated from pKOS232-172 as an 18 kb NdeI/HindIII fragment and the srmG3,4,5 cassette was isolated from pKOS232-182 as a 20 kb HindIII/AvrII fragment. These fragments plus a pSET152-based vector having the tylG promoter (the vector portion gel isolated from pKOS244-20) were joined in a three-piece ligation and recombinants were recovered by in vitro lambda phage packaging and infection of *E. coli*. Correct constructs were identified by restriction analysis (pKOS232-184A) and transferred into *E. coli* DH5α/pUB307.

**[0120]** The resulting *E. coli* DH5a/pUB307 cells were conjugated with *S. fradiae* K159-1/244-17a (Example 3) to produce *Streptomyces fradiae* K232-192. *Streptomyces fradiae* K232-192 was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 12 March 2003, with accession number PTA-5052. Conjugation was performed as described in Practical Streptomyces Genetics (Kieser et al., 2000) except that plates were left overnight at 37°C before overlaying with the selective agent (apramycin and naladixic acid). Apramycin resistant exconjugants were streaked for single colonies and a set of clones were patched onto R5 plates and inoculated into tryptic soy broth (40 ml in 250 ml shake flasks). Both the solid and liquid media contained apramycin (to select for pKOS232-184A) and kanamycin (to select for pKOS244-17A). Liquid and solid cultures were grown at 30°C. Agar plugs taken from most patches on R5 showed bioactivity when placed on an *M. luteus* test lawn. The agar was extracted with ethyl acetate and found to contain a compound of 730 amu. TSB seed cultures at 2-3 days were used to inoculate fermentation media and these cultures were grown for 7-10 days at 28°C. Upon extraction with ethyl acetate the 730 amu compound (730-I) was isolated and its structure verified by NMR as shown below. In addition, LC-MS analysis of the filtered culture broth showed abundant production of a 586 amu and a 730 amu (730-II) compound, and a 714

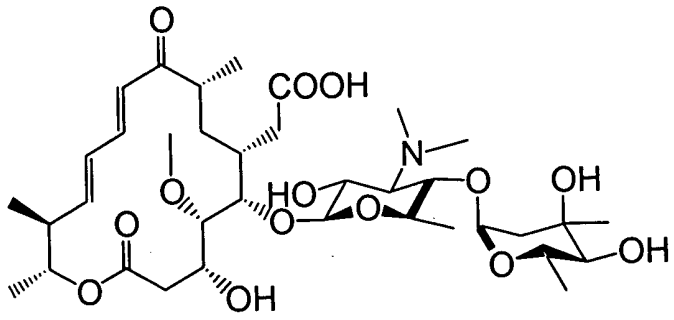
amu compound and a smaller amount of a 904 amu compound (most likely representing 14-methyl-platenolide with all three sugars attached). Thus, the chalcomycin-spiramycin hybrid PKS synthesized the predicted 14-methyl platenolide.



730 amu (730-I)

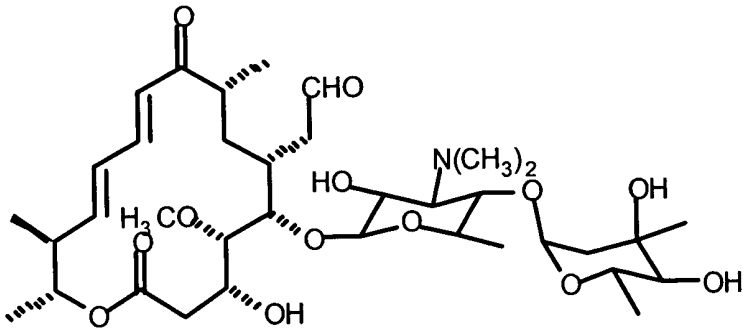


586 amu

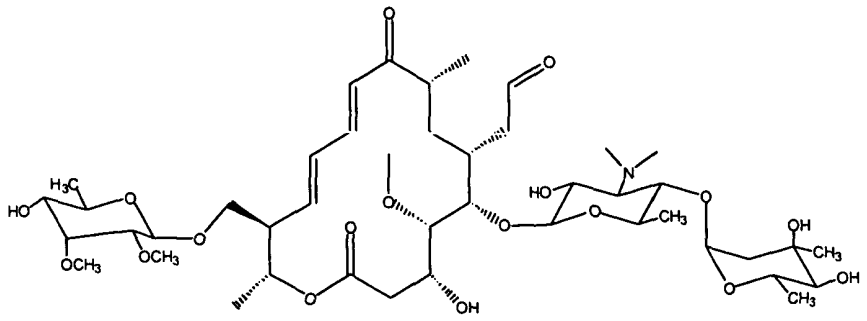


730 amu

**(730-II)**



714 amu



904 amu

Example 6 Construction of *Streptomyces fradiae* K344-51 Expressing a Hybrid Chalcomycin-Spiramycin PKS and the *chmH* Gene

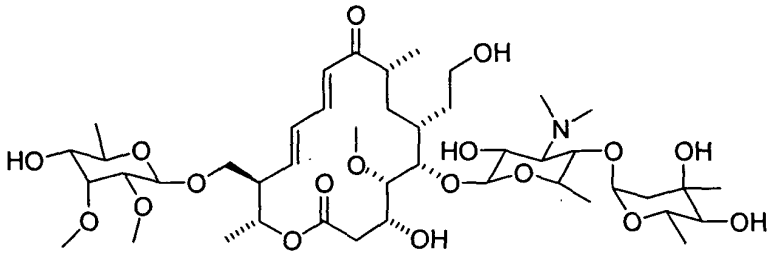
**[0121]** The *chmH* gene was cloned from cosmid pKOS146-185.1, which was deposited under the terms of the Budapest Treaty with the American Type Culture Collection, 10801 University Blvd., Manassas, VA, 20110-2209, on 19 February 2003, with accession number PTA-4961.

**[0122]** A 6.3 Kb EcoRI fragment containing the *chmH* gene and a small downstream ferredoxin gene was cloned from cosmid pKOS146-185.1 into Litmus28 to give pKOS344-10B. This was then cut with SacI and religated to give pKOS344-016 having a 2.1 Kb insert. An NdeI site was introduced at the start of translation and an internal NdeI site was simultaneously replaced with a PstI site (without changing the amino acid sequence) in a three-piece ligation using two PCR products ligated between the EcoRI and BamHI sites of pKOS344-016 to give pKOS344-022B. The unique FseI site in pKOS344-022B was changed to an XbaI site with a synthetic linker and the *chmH* gene plus ferredoxin gene were excised with NdeI and XbaI and ligated into the expression vector, pKOS342-108D, between the NdeI and AvrII sites to give pKOS344-037B. This vector was transferred into DH5α/pUB307, and conjugated into K232-192 (Example 5). Exconjugants were selected with thiostrepton and streaked for single colonies to yield *S. fradiae* K344-51.

**[0123]** The vector for integration of *chmH*, pKOS342-108D, uses the *int* and *att* functions of *Streptomyces* phage ϕBT1. All *S. fradiae* strains were plated on AS1 agar for sporulation, R5

agar for solid media production, or grown in liquid TSB for vegetative growth and Russia medium for production. All appropriate antibiotics for selection of integrated markers were added to the media, except for the production stage.

**[0124]** Expression of the chmH gene (with its downstream ferredoxin) dramatically increased hydroxylation of the 14-methyl. A 906 amu compound was detected. This is the structure expected when all post-PKS tailoring of the tylosin pathway occurred, along with an additional reduction adding two hydrogen atoms. The methanol adduct characteristic of the aldehyde is not seen for the 906 amu compound, and reduction of the aldehyde most likely accounts for the addition of the two hydrogen atoms.



**906 amu**

**[0125]** In addition, there appear to be a significant amount of 730 amu (730-I) compound that has the aldehyde and is hydroxylated on the 14-methyl. This is deduced by the presence of the methanol adduct (762 amu) and the fact that the 730 amu compound now elutes later from the C18 column compared with the 730 amu carboxylic acid seen prior to expression of *chmH*.

**[0126]** Without intending to be bound by a specific mechanism, Figure 2 shows proposed pathways for post-PKS modification of the chalcomycin-spiramycin hybrid PKS macrolide product in the absence or presence of ChmH. When ChmH is present, the post-PKS reaction sequence from the Chm/Srm hybrid essentially follows that for tylosin and gives the 904 amu structure, which is converted by reduction of the aldehyde to a 906 amu compound. This reduction of the aldehyde has been described for tylosin (to give relomycin). Knockout of genes for allose biosynthesis or its transfer (tylJ), would give the demycinosyl compound of 730 amu (with the 14-hydroxymethyl and the aldehyde).

Example 7 Expression of a *chmGI-GII* operon with the *tylG2* C-terminal linker in *S. fradiae* K105-2

a) Construction of the *S. fradiae tylD* knockout strain K168-173

**[0127]** The *tylD* knockout plasmid was constructed from two PCR products encompassing 1.8 kb regions upstream and downstream of the *tylD* gene using PCR primers that introduced new restriction sites. The upstream PCR product was cut with EcoRI and PstI and the downstream product was cut with PstI and SphI. These were then ligated together between the EcoRI and SphI sites of pUC19 and the sequence was verified. The resulting plasmid, pKOS168-106, has about 80% of the *tylD* gene deleted between the artificial PstI sites. This plasmid was introduced into *S. fradiae* by conjugation from *E. coli* DH5α/pUB307 and apramycin resistant exconjugants were obtained. Three were found by PCR to be the result of homologous recombination at the expected *tylD* locus and these were grown in the absence of selection and screened for the second crossover. Apramycin sensitive clones were isolated and some were found that produced demycinosyltylosin (DMT) by LC-MS analysis of the fermentation broths. The strain was designated *S. fradiae* K168-173.

b) Construction of *S. fradiae tylD* K105-2

**[0128]** The *S. fradiae* DMT (demycinosyltylosin) producer (K168-173) described above was used to introduce a KS-1 null mutation in the tylosin PKS. The plasmids pKOS168-190 and pKOS268-145 were digested with EcoRI and EcoRV and the 6.2kb and 2.6kb fragments, respectively, were gel isolated and ligated together to give pKOS264-65. A mutation was introduced into pKOS264-65 using PCR to change the active site cysteine of the tylosin KS1 to alanine, with the simultaneous introduction of an NheI site, to give pKOS325-8. Finally, pKOS325-8 and pKOS241-52 were digested with PvuII and XbaI and ligated together to give pKOS264-76. Plasmid pKOS264-76 was conjugated into the DMT producer strain *S. fradiae* K168-173 (Example 5) from *E. coli* DH5α/pUB307 and exconjugants were selected for apramycin resistance. Clones that underwent the correct first crossover event were identified by Southern blot analysis and one of these was propagated without selection to allow a second crossover. DNA from clones that had become apramycin sensitive was digested with XmaI/NheI and analyzed by Southern blot. Three clones had the pattern consistent with that expected for the desired second crossover to leave the KS1-null mutation in the chromosome. This strain was

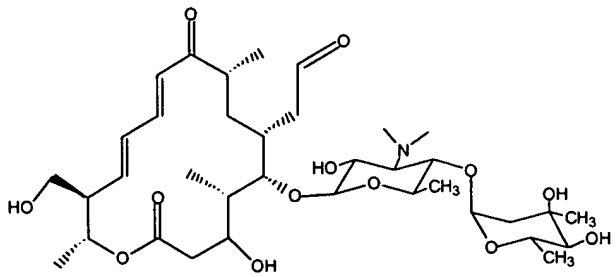
designated *S. fradiae* K105-2, and was shown to produce no tylosin-related structure, but could convert O-mycaminosyl-tylonolide (OMT) into demycinosyl-tylosin (DMT).

c) Construction of a *chmGI-GII* operon with the *tylG2* C-terminal linker

**[0129]** The *tylG2* C-terminal linker region was isolated by PCR from a pSET-based vector encoding the including the entire tylosin PKS (pKOS168-190). The primers used were TylLink-A: 5'-TGAAGCTTCCCGCCACGCTGGTG-3' [SEQ ID NO:__] and TylLink-B: 5'-CGTCTAGACAGGGTGTGAAACCG-3') [SEQ ID NO:__]. This created a HindIII site at the same position of the encoded sequence corresponding to the natural HindIII site in the linker region of *chmGII*. The amplimer was cut with HindIII and XbaI and ligated into Litmus29 to give pKOS342-78. The tylosin linker region of pKOS342-78 was excised using HindIII and XbaI, and then ligated into HindIII and XbaI-digested pKOS232-172 (Example 5) to create pKOS342-82. This hybrid piece was then cut out with NdeI and XbaI and ligated to a pSET-based vector (pKOS232-189) cut with NdeI and SpeI to create the pSET152-based expression vector, pKOS342-84.

d) Expression of a *chmGI-GII* operon with the *tylG2* C-terminal linker in *S. fradiae* K105-2

**[0130]** The expression vector pKOS342-84 was transferred to *E. coli* DH5α/pUB307 and conjugated into *S. fradiae* K105-2 (this example, above) . Apramycin resistant colonies were isolated and fermented in production medium. The broth was analyzed by LC-MS and found to contain the compound shown below. The chm/tyl hybrids differ from the chm/srm hybrids only by having a 4-methyl in place of a 4-methoxy, apparently making the chm/tyl good substrates for the TylH hydroxylase.




714 amu

***

**[0131]** All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference.

**[0132]** Although the present invention has been described in detail with reference to specific embodiments, those of skill in the art will recognize that modifications and improvements are within the scope and spirit of the invention, as set forth in the claims which follow. Citation of publications and patent documents is not intended as an admission that any such document is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description are for purposes of illustration and not limitation.